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# Nucleoside Diphosphate Prodrugs: Nonsymmetric Di*PP*ro-Nucleotides

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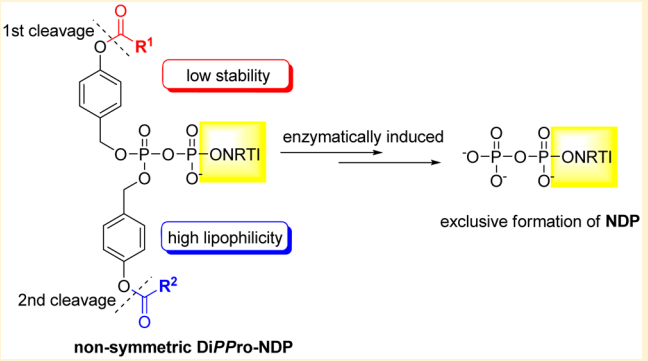
Supporting Information

**ABSTRACT:** Nonsymmetric Di*PP*ro-nucleotides are described as nucleoside diphosphate (NDP) delivery systems. The concept is to attach different bis(acyloxybenzyl) moieties at the $\beta$-phosphate moiety of a NDP. Di*PP*ro compounds bearing two alkanoylbenzyl residues and Di*PP*ro compounds bearing an alkanoylbenzyl or a benzoylbenzyl group as bioreversible prodrug moieties were studied. Compounds bearing short chain alkanoyl esters led to a fast hydrolysis by chemical or enzymatic means. The ester group in the second prodrug group comprised a long lipophilic aliphatic or an aromatic residue. The lipophilicity of this group enabled the prodrug to penetrate the cell membrane. The introduction of two different groups allowed a controlled stepwise removal of the prodrug moieties to achieve a highly selective delivery of the NDP in CEM cell extracts. The compounds were highly active against HIV even in thymidine kinase-deficient CEM cells. Thus, the compounds, although charged at the $\alpha$-phosphate group, were taken up by the cells and released NDPs.




## INTRODUCTION

Nucleoside analogues are extensively used as agents in antiviral and antitumor chemotherapy. However, their biological activity is strongly dependent on their intracellular activation by host cell kinases to give, via the mono- and the diphosphate, the ultimately active triphosphate derivative which inhibits the function of the viral DNA polymerase. However, in the case of nucleoside analogues cellular kinases often catalyze these metabolic steps insufficiently.[1,2] The result might be a partial loss of antiviral activity or for some nucleoside analogues even a complete failure to exhibit any antiviral activity. Moreover, inefficient phosphorylation led in some cases to adverse effects.[3,4] The use of prodrug forms of phosphorylated nucleoside analogues (pronucleotides) can circumvent rate limiting steps within the metabolic pathway by bypassing at least one, if not several, involved activating enzymes.[5,6] This task has been successfully achieved in the past for the intracellular delivery of monophosphates of nucleoside analogues using prodrug strategies such as the *cyclo*Sal-,[7−9] Sate-,[10,11] bisPOM-[12] and phosphoramidate-nucleotide approaches.[13,14]

Remarkably, in contrast to numerous examples of successful nucleoside monophosphate prodrug approaches for the bypass of nucleoside kinases, the development of nucleoside diphosphate (NDP) prodrugs has been very rarely addressed. However, such a prodrug approach might be desirable, for instance, in the case of 3′-azido-3′-deoxythymidine (AZT), which is very poorly phosphorylated from AZTMP to yield AZTDP by thymidylate kinase (TMP-K) in human cells.[15,16]

Hostetler et al. synthesized different nucleoside diphosphate diglycerides as potential NDP prodrugs.[17−20] However, the hydrolysis of these compounds delivered the monophosphates instead of the diphosphates as a result of the cleavage of the diphosphate moiety. A second approach was reported by Huynh-Dinh et al.[21−23] In their approach, the $\beta$-phosphate was acylated with fatty acids forming a mixed anhydride bond. In chemical hydrolysis studies the NDP was formed. However, in cell extracts an undefined decomposition of the compounds was observed.

Since a few years, we turned our interest toward the bioreversible protection of nucleoside diphosphates although, as compared to nucleoside monophosphate prodrugs, the design of such compounds is more demanding because of a chemical reason. In NDPs, a complete lipophilic modification of the charged and therefore highly polar diphosphate unit led to a considerable decrease in chemical stability of this part of the molecule. As a result, the phosphate anhydride bond of the nucleoside diphosphate was cleaved leading to two monophosphorylated fragments.

Earlier, we reported on the Di*PP*ro approach. In this approach, esterification of the $\beta$-phosphate of a NDP with two

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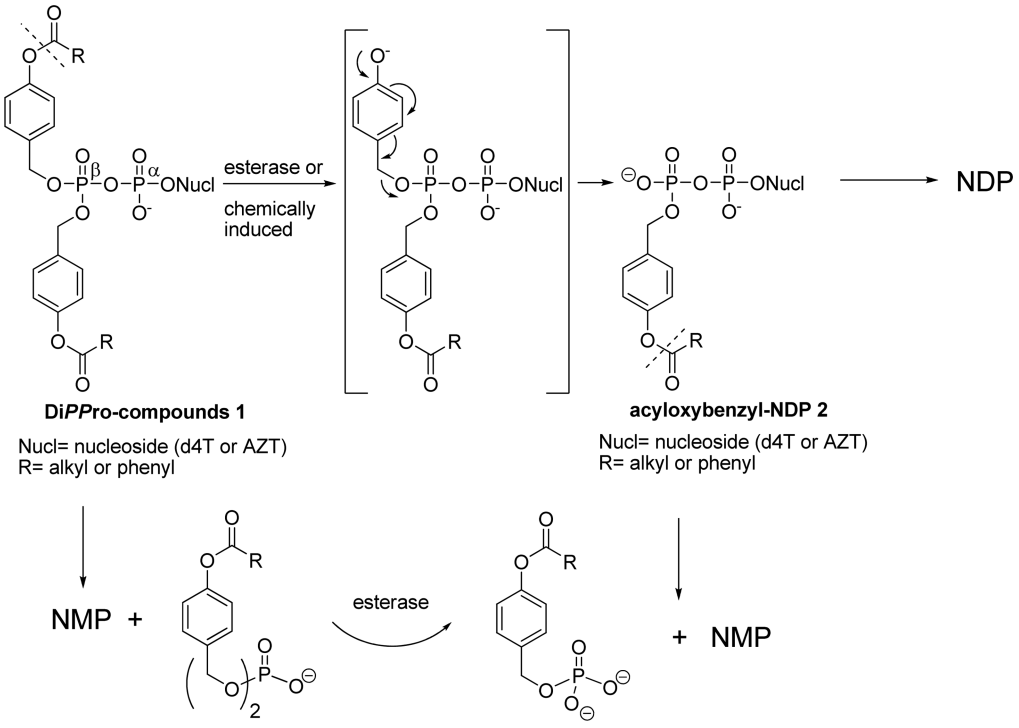
**Scheme 1. Hydrolysis of earlier reported symmetric bis(acyloxybenzyl)-Di*PP*ro compounds 1.**




bis(acyloxybenzyl) moieties neutralizes the charges at this phosphate group. In previous studies this was achieved by masking the $\beta$-phosphate using two identical bis(alkanoyloxybenzyl)[24,25] or bis(benzoyloxybenzyl) moieties[26] (symmetrical modification, $R^1 = R^2$). The concept was based on an enzyme-driven cleavage of these masking units, which ensured that the release of NDPs preferably took place intracellularly because of the higher concentrations of the esterases/lipases in the target cells. In contrast to the low stability in the presence of esterases, the chemical stability at physiological pH was still found to be high. By choosing the appropriate substituent R in the ester moiety within the mask, it was possible to "tune" both stability and lipophilicity of the prodrugs (Scheme 1).[25]

Although we were able to prove that this approach works, an unwanted property of the symmetric aliphatic or aromatic Di*PP*ro compounds was that also some nucleoside monophosphate (NMP) was detected in addition to the wanted nucleoside diphosphate, e.g., in PBS solution, pH 7.3, or cell extract hydrolysis studies. Interestingly, it was shown that the amount of NMP clearly correlated with the stability of the Di*PP*ro compounds and that the stability of the compounds was dependent on the length of the alkyl residues R attached via an ester linkage to the 4-hydroxybenzyl group: the longer the alkyl group R, the higher was the stability of the Di*PP*ro compounds but the higher was also the amount of NMP formed in the chemical hydrolyses and in the cell extract studies. As the reason for the formation of NMP, a concurrence reaction was identified in which water/hydroxide reacted at the phosphorus atom of the $\beta$-phosphate group. This led to the cleavage of the phosphate anhydride bond releasing the NMP. This side reaction became more relevant with increasing stability of the ester linkage in the prodrug moiety. On the other hand, we observed that after the removal of the first mask to give a monomasked intermediate such as **2**, no further increase of the amount of NMP was observed (Scheme 1). The reason here was that after the first removal an additional charge appeared at the $\beta$-phosphate, and this obviously prevented a nucleophilic reaction at this moiety.

Taking all previous results together, these findings led to the development of a second generation of Di*PP*ro compounds. Here, we disclose a study on a series of *nonsymmetric* Di*PP*ro-nucleotides **1** bearing two different masking units ($R^1$ not equal to $R^2$). The design was the following: One masking group contained a short alkyl chain carboxylic acid ester which, because of our previously reported data,[25] will be rapidly cleaved by chemical or enzymatic means. The second masking group contained a long alkyl residue carboxylic acid ester or a substituted benzoic acid ester, which adds high lipophilicity to the molecule. It was expected that such a construct would allow a rapid conversion of the Di*PP*ro compound **1** into the monomasked intermediate **2** and thereby should avoid the side reaction to form the unwanted nucleoside monophosphate. The second mask will be cleaved subsequently from the intermediate to form the nucleoside diphosphate. It was expected that with these nonsymmetric compounds a highly selective conversion of the Di*PP*ro compounds into nucleoside diphosphates can be achieved.

## RESULTS AND DISCUSSION

**Synthesis.** For the synthesis of nonsymmetric Di*PP*ro-NDPs **1**, we first tried to use the protocol of the symmetric counterparts.[24−26] According to that protocol, an amount of 2 equiv of an appropriate 4-acyloxybenzyl alcohol was reacted with dichloro-*N*,*N*-diisopropylaminophosphoramidite to give the corresponding phosphoramidite like **5** ($R^1 = R^2$) which was then coupled by an acid-mediated reaction with a nucleoside monophosphate followed by oxidation. For the new nonsymmetric compounds an attempt was made to introduce two different 4-acyloxybenzyl masking units **3** and **4** by the same way. However, this approach failed.



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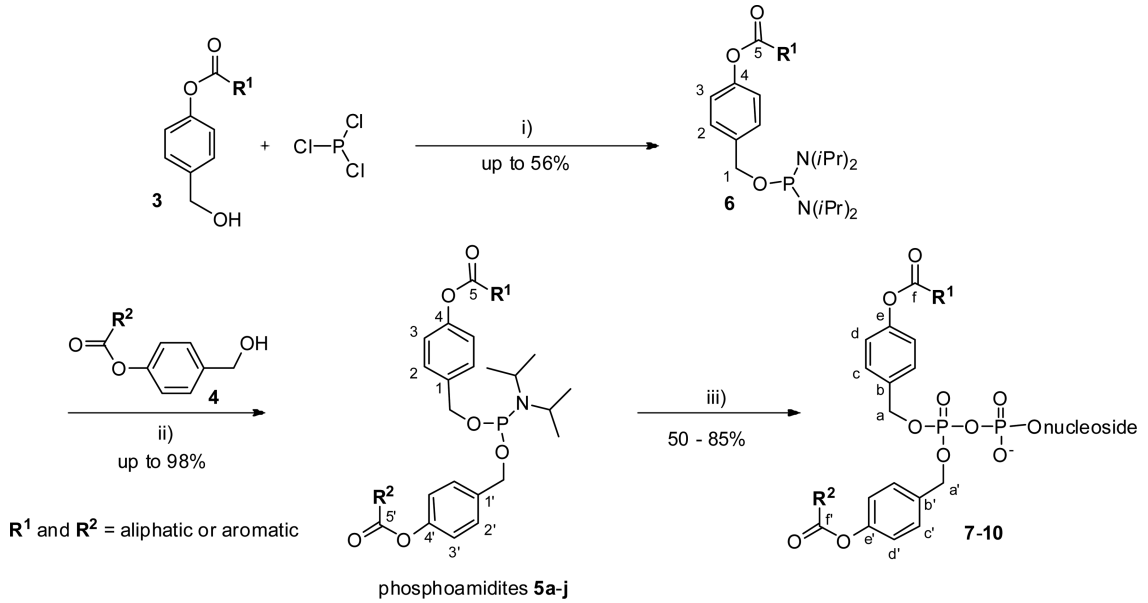
**Scheme 2. Synthesis of Nonsymmetric Di*PP*ro-NDPs 7−10**[a]




[a](i) (a) 1 equiv of **3**, 1 equiv of pyridine, −78 °C to rt, 24 h, THF; (b) 6.1 equiv of *N*,*N*-diisopropylamine, −10 °C to rt, 24−48 h, THF; (ii) 0.67 equiv of **4**, 0.67 equiv of DCI-activator solution (0.25 M in acetonitrile), 4 °C to rt, 30−60 min, acetonitrile; (iii) (a) 1 equiv of $[N(nC_4H_9)_4]$d4TMP or $[N(nC_4H_9)_4]$AZTMP, 1.5 equiv of **5**, 1.25−1.75 equiv of DCI-activator solution (0.25 M in acetonitrile), rt, 30 min, acetonitrile; (b) 1.5 equiv of *t*-BuOOH solution (5.5 M in *n*-decane), rt, 15 min, acetonitrile. Indices shown in compound **5**, **6**, and **7**−**10** are used for NMR assignment in Experimental Section.

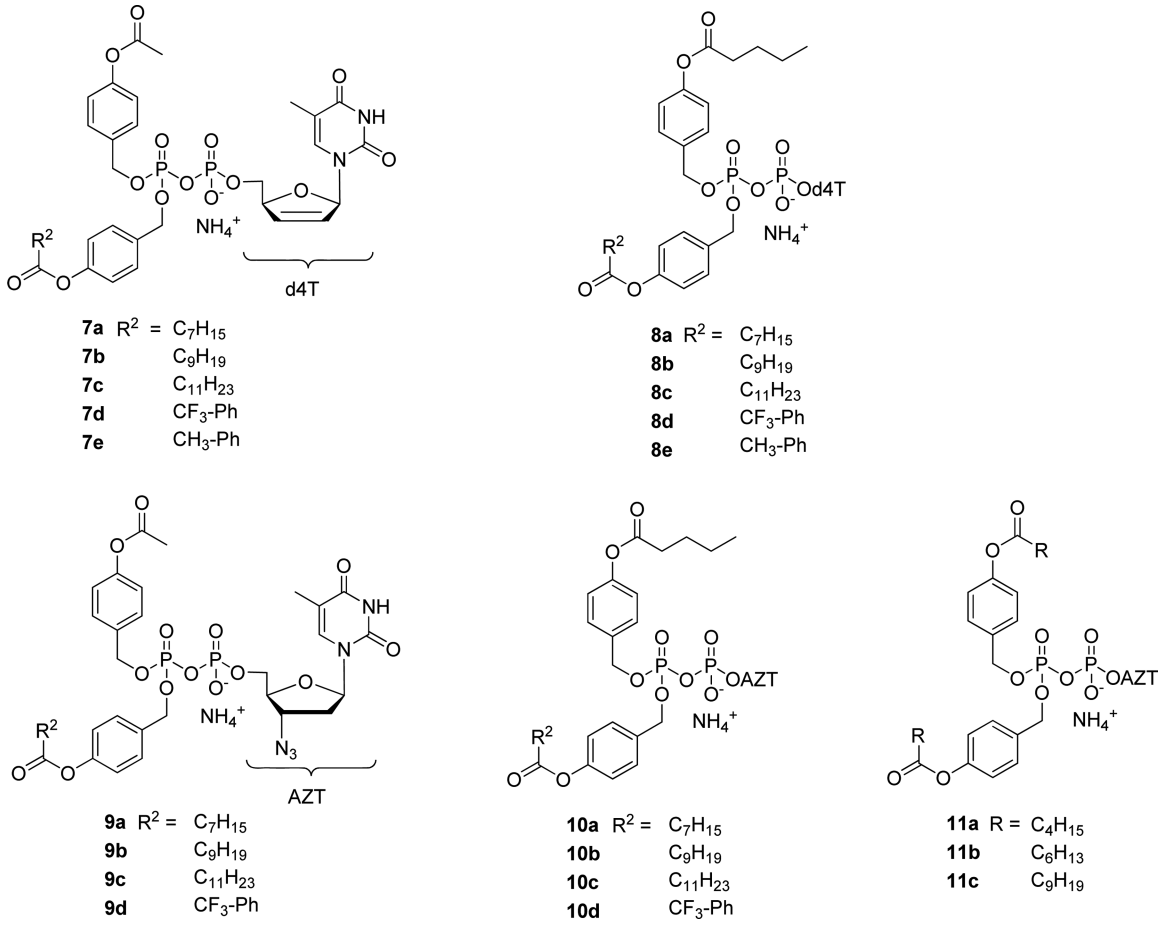



**Figure 1.** Nonsymmetric Di*PP*ro-d4TDPs **7**, **8** and Di*PP*ro-ATZDPs **9**, **10**.



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**Table 1. Half-Lives of Di*PP*ro-d4TDPs 7, 8 and Di*PP*ro-AZTDPs 9, 10 in PBS and CEM/0 Cell Extracts**

| | NDP = d4TDP 7 | | NDP = AZTDP 9 | |
|---|---|---|---|---|
| compd | $t_{1/2}$ [h] in PBS | $t_{1/2}$ [h] in CEM/0 | $t_{1/2}$ [h] in PBS | $t_{1/2}$ [h] in CEM/0 |
| $CH_3/C_7H_{15}$-Di*PP*ro-NDP **7a**/**9a** | 36 | 0.04 | 33 | 0.02 |
| $CH_3/C_9H_{19}$-Di*PP*ro-NDP **7b**/**9b** | 40 | 0.04 | 35 | 0.04 |
| $CH_3/C_{11}H_{23}$-Di*PP*ro-NDP **7c**/**9c** | 48 | 0.16 | 32 | 0.11 |
| $CH_3/CF_3$-Ph-Di*PP*ro-NDP **7d**/**9d** | 15 | 0.03 | 13 | 0.04 |
| $CH_3/CH_3$-Ph-Di*PP*ro-NDP **7e** | 58 | 0.04 | | |
| | NDP = d4TDP **8** | | NDP = AZTDP **10** | |
| compd | $t_{1/2}$ [h] in PBS | $t_{1/2}$ [h] in CEM/0 | $t_{1/2}$ [h] in PBS | $t_{1/2}$ [h] in CEM/0 |
| $C_4H_9/C_7H_{15}$-Di*PP*ro-NDP **8a**/**10a** | 36 | 0.71 | 46 | 0.83 |
| $C_4H_9/C_9H_{19}$-Di*PP*ro-NDP **8b**/**10b** | 40 | 1.05 | 50 | 1 |
| $C_4H_9/C_{11}H_{23}$-Di*PP*ro-NDP **8c**/**10c** | 52 | 1.91 | 63 | 1.65 |
| $C_4H_9/CF_3$-Ph-Di*PP*ro-NDP **8d**/**10d** | 15 | 0.88 | 19 | 0.83 |
| $C_4H_9/CH_3$-Ph-Di*PP*ro-NDP **8e** | 50 | 0.96 | - | - |

Finally, in a new protocol 4-acyloxybenzyl alcohol **3** was reacted with phosphorus trichloride followed by the addition of 2 equiv of *N*,*N*-diisopropylamine (DIPA) which led to bis(diisopropylamino)phosphoramidites **6** in yields up to 56% in a *one-pot* reaction. Then, one of the diisopropylamino moieties was replaced by the second 4-acyloxybenzyl alcohol **4** after activation with dicyanoimidazol (DCI) to yield the nonsymmetric phosphoramidites **5** in very high yields.

Next, a second acid-activated coupling of these phosphoramidites **5** with the corresponding nucleoside monophosphates was carried out. At the beginning, the conversions obtained in this coupling reaction were surprisingly low, which was proven by NMR and HPLC analyses of the crude mixtures. Changing the equivalents of phosphoramidite **5** and the DCI activator did not led to an improvement. Finally, the problem was solved by the successive addition of small amounts of the DCI-activator solution. Starting with the addition of 0.5 equiv of DCI-activator solution to a solution of 1 equiv of the nucleoside monophosphate and 1.5 equiv of the phosphoramidite **5**, followed by the stepwise addition of 0.25 equiv every 5 min up to a total of 1.25 or 1.75 equiv of DCI led to a quantitative conversion of the nucleotide. Di*PP*ro-nucleotides **7**−**10** were successfully isolated by using automated flash RP-18-chromatography. By use of the new protocol, numerous combinations of Di*PP*ro-nucleotides were accessible in quantitative conversions, high isolated yields, and high purity, which was checked by means of $^1$H, $^{13}$C, and $^{31}$P NMR spectroscopy, mass spectrometry, and HPLC analysis. The route for the synthesis of nonsymmetric Di*PP*ro-NDPs **7**−**10** is summarized in Scheme 2.

Structural formulas of Di*PP*ro-d4TDPs **7**, **8** and Di*PP*ro-AZTDPs **9**, **10** bearing different aliphatic ($R^2 = C_nH_{2n+1}$) and aromatic ($R^2$ = X-Ph) acyl moieties as the lipophilic masks in combination with short aliphatic acyl moieties ($R^1$= $CH_3$, $C_4H_9$) in the acyloxybenzyl-masking group are summarized in Figure 1. Also three symmetric Di*PP*ro-AZTDPs **11a**−**c** were prepared by the earlier published method.

**Hydrolysis Studies.** All prepared compounds were studied with regard to their stabilities and their hydrolysis products in different media.

*Chemical Stability in Phosphate Buffer, pH 7.3.* We expected that the more labile mask would be cleaved faster to form intermediate **2** bearing the long, lipophilic, and hydrolytically more stable mask. However, surprisingly in the chemical hydrolysis studies in most of the cases both intermediates were observed by means of HPLC analysis. In the monomasked intermediates **2**, the short alkanoyl ester moieties were more rapidly hydrolyzed as compared to the long alkyl bearing moieties. Furthermore, the half-lives determined in phosphate buffer, pH 7.3, of the nonsymmetric Di*PP*ro compounds were surprisingly higher as compared to those of the symmetric Di*PP*ro-NDPs. This can be seen for example for compound **7b** ($CH_3/C_9H_{19}$-Di*PP*ro-d4TDP). In this case the half-life of the cleavage of the first masking group bearing the acetate group was 40 h while the same masking group in the symmetrical di-$CH_3$-Di*PP*ro-d4TDP was cleaved with a half-life of 10 h only.[24] Steric hindrance or aggregation due to the second, more lipophilic moiety might be a reason for this increase in stability. The determined half-lives of the formation of the monomasked intermediate were between 15 and 69 h as summarized in Table 1.

The chemical stabilities of Di*PP*ro-AZTDPs **9**, **10** were in the same range as those of the Di*PP*ro-d4TDPs **7**, **8** having identical prodrug moieties. Only $CH_3/C_{11}H_{23}$-Di*PP*ro-AZTDP **9c** showed a lower stability compared to the corresponding d4TDP derivative **7c** for some unknown reasons. Even in comparison with the Di*PP*ro compounds with shorter alkyl chains the stability of $CH_3/C_{11}H_{23}$-Di*PP*ro-AZTDP **9c** was markedly low. Nevertheless, all Di*PP*ro compounds **7**−**10** hydrolyzed in buffer to form almost exclusively the nucleoside diphosphate.

As reported before, some NMP was formed as well probably because of the unexpected high hydrolytic stability compared to the symmetric Di*PP*ro compounds bearing short alkyl chains in the masking group. It is important to mention that the ratio of NDP to NMP in these chemically driven hydrolyses was higher as described for the symmetric compound with longer chains.[24,25]

We also evaluated the chemical stability of Di*PP*ro-AZTDP **9b** and **10b** in Roswell Park Memorial Institute (RPMI) medium in addition to PBS solution, pH 7.3, as described above. Both compounds showed also under these conditions high stability ($t_{1/2}$ = 19 h for **9b** and $t_{1/2}$ = 77 h for **10b**) and were found to be markedly more stable as compared to the CEM/0 cell extracts.

*Incubations in Cell Extracts of Wild Type $CD_4^+$ T-Lymphocyte Cells (CEM/0 Cell Extracts).* As in the above-described studies in PBS and RPMI culture medium, the half-lives of the Di*PP*ro compounds were found to be independent of the nucleoside analogue attached to the molecule. In contrast to the chemical hydrolysis studies, the prodrugs delivered the corresponding nucleoside diphosphate selectively within 2−7 h



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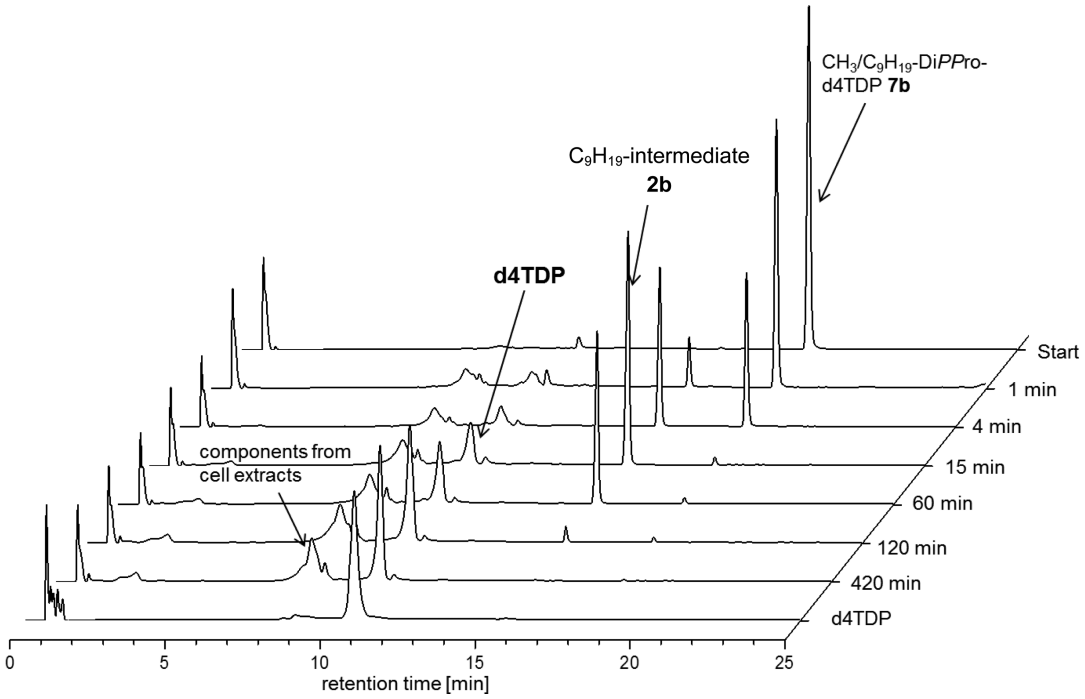


**Figure 2.** RP-18 HPLC chromatograms of the hydrolysis of $CH_3/C_9H_{19}$-Di*PP*ro-d4TDP **7b** in CEM/0 cell extracts.

only. In the case of the $CH_3/R^2$-Di*PP*ro-NDPs **7** and **9** the hydrolysis led exclusively to the intermediates bearing the long lipophilic acyl group with half-lives of minutes (Table 1). In all cases the acetate ester was selectively cleaved first. However, in comparison the cell extract hydrolysis of the $C_4H_9/R^2$-Di*PP*ro-NDPs **8** and **10** released in addition to the intermediate bearing the long alkyl chain acyl group also the $C_4H_9$ intermediate, although in a markedly smaller ratio. This is due to the higher stability of the pentanoyl ($R^1 = C_4H_9$) ester moiety compared to the acetate group present in compounds **7** and **9**. But also in these cases the diphosphate was finally formed from both intermediates selectively. The more lipophilic mask had no influence on the half-lives of the cleavage of the short chain acyl group ($R^1 = CH_3$ and $R^1 = C_4H_9$, respectively, Table 1) except for the four Di*PP*ro-nucleotides with $R^2 = C_{11}H_{23}$ (**7c−10c**) which showed different stabilities. Figure 2 summarizes as an example the hydrolysis of $CH_3/C_9H_{19}$-Di*PP*ro-d4TDP **7b** in CEM/0 cell extracts followed by means of RP-18 HPLC. After 60 min virtually all of the prodrug **7b** was consumed. Only the $C_9H_{19}$ intermediate **11b** and d4TDP appeared as hydrolysis products at that time point. Whether traces of d4TMP were also formed remained unclear because the detection of d4TMP was not possible by RP-18 HPLC because of remaining cell extract components that eluted in the chromatogram with identical retention time. On the other hand, by use of a HILIC column (HILIC: hydrophilic interaction liquid chromatography) in the chromatography, the separation of d4TMP from cell extract components was successful. With this second analytical method it was proven that very small amounts of d4TMP were also present in the CEM cell extract hydrolysis samples of the Di*PP*ro compounds.

In order to study if the detected d4TMP resulted either from the side reaction described above or from a dephosphorylation of d4TDP to give d4TMP due to phosphatases present in the extracts, both d4TDP and AZTDP were incubated as well in the CEM/0 cell extracts and aliquots of the hydrolysis mixtures were analyzed after 2, 4, 7, and 10 h of incubation. In both cases d4TMP and AZTMP were detected. The half-life of dephosphorylation of d4TDP was 10 h, while that of AZTDP was 19 h in the experimental setup. For this reason, we concluded that the trace amounts of the nucleoside monophosphates found in the above-described hydrolysis studies of the Di*PP*ro compounds in CEM/0 cell extracts were most likely a result of a dephosphorylation of the formed diphosphates from the prodrugs.

In the case of the $C_4H_9/R^2$-Di*PP*ro-nucleotides **8** and **10** the hydrolysis proceeded via both possible intermediates and then led to the corresponding NDPs. As the half-lives for the starting Di*PP*ro compounds **1** bearing an acetyl ($R^1 = CH_3$) or a pentanoyl ($R^1 = C_4H_9$) residue, the half-lives for the formed monomasked intermediates of type **2** were dependent on the acyl ester group. The half-lives of monomasked intermediates were determined by the decrease of the corresponding peak areas in the HPL-chromatograms of these compounds in the hydrolysis mixtures of the $CH_3/R^2$-Di*PP*ro-nucleotides **7** and **9** in CEM/0 cell extracts after full consumption of the starting Di*PP*ro compounds. The results are summarized in Table 2.

We assumed that the stability would increase with longer chain length. Interestingly, intermediates **2a** and **2′a** were more stable than the ones with longer chain length. Therefore, it

**Table 2. Half-Lives of the Intermediates 2 and 2′ in CEM/0 Cell Extracts**

| | $t_{1/2}$ [min] in CEM/0 | |
|---|---|---|
| intermediate (with $R^2$) | **2** NDP = d4TDP | **2′** NDP = AZTDP |
| $C_7H_{15}$ **2a/2′a** | 99 | 99 |
| $C_9H_{19}$ **2b/2′b** | 50 | 50 |
| $C_{11}H_{23}$ **2c/2′c** | 69 | 63 |
| Ph-$CF_3$ **2d/2′d** | 690 | 230 |
| Ph-$CH_3$ **2e** | 870 | |



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seemed that the octanoyl group was a worse substrate for the ester cleaving enzymes.

In the case of the $CF_3$-Ph intermediate **2d** it is surprising that the attached nucleoside had an influence on the stability. The AZT intermediate **2′d** was markedly more labile than the d4T intermediate **2d** for unknown reasons.

*Incubations with Pig Liver Esterase.* The nonsymmetric aliphatic Di*PP*ro-AZTDPs **9a−c** and **10a−c** bearing a $R^1 = CH_3$ or $C_4H_9$ in combination with long alkyl chains as the $R^2$ residue were incubated with pig liver esterase (PLE) to investigate the influence of the chain length on the enzymatic cleavage by this esterase. Furthermore, $CH_3/C_9H_{19}$-Di*PP*ro-d4TDP **7b** and $C_4H_9/C_9H_{19}$-Di*PP*ro-d4TDP **8b** were also included to study whether the attached nucleoside analog has an effect on the hydrolysis pathway or on the compound stability.

The results of the hydrolysis experiments in the presence of PLE are summarized in Table 3.

**Table 3. Half-Lives of the Di*PP*ro Compounds 7−10 in the Presence of PLE**

| | $t_{1/2}$ [min] of | |
|---|---|---|
| compd | Di*PP*ro-NDP | $R^1$ intermediate |
| $CH_3/C_9H_{19}$-Di*PP*ro-d4TDP **7b** | 0.14 | nm.[a] |
| $CH_3/C_7H_{15}$-Di*PP*ro-AZTDP **9a** | 0.26 | nm.[a] |
| $CH_3/C_9H_{19}$-Di*PP*ro-AZTDP **9b** | 0.21 | nm.[a] |
| $CH_3/C_{11}H_{23}$-Di*PP*ro-AZTDP **9c** | 0.45 | nm.[a] |
| $C_4H_9/C_9H_{19}$-Di*PP*ro-d4TDP **8b** | 0.19 | 28 |
| $C_4H_9/C_7H_{15}$-Di*PP*ro-AZTDP **10a** | 0.27 | 15 |
| $C_4H_9/C_9H_{19}$-Di*PP*ro-AZTDP **10b** | 0.27 | 19 |
| $C_4H_9/C_{11}H_{23}$-Di*PP*ro-AZTDP **10c** | 1.2 | 38 |

[a]nm.: not measurable.

All compounds were rapidly hydrolyzed and delivered the nucleoside diphosphates d4TDP or AZTDP exclusively within a few minutes. The half-lives determined for the $CH_3/C_7H_{15}$ and $CH_3/C_9H_{19}$ compounds **9a,b** as well as the $C_4H_9/C_7H_{15}$ and $C_4H_9/C_9H_{19}$-masked pronucleotides **10a,b** were found to be all in the same range (0.21−0.27 min). The influence of the nucleoside on the half-live was small, although Di*PP*ro-d4TDPs **7b** and **8b** proved to be somewhat less stable. In contrast, both compounds **9c**, and **10c** ($CH_3/C_{11}H_{23}$- and $C_4H_9/C_{11}H_{23}$-Di*PP*ro-AZTDP) bearing the longest aliphatic chain showed higher stability against hydrolysis by PLE.

Remarkably, in the case of the hydrolysis of all $CH_3/R^2$-Di*PP*ro **9a−c**, **7b**, exclusively the hydrolysis intermediate bearing the long aliphatic chain was formed although in very small amounts. Therefore, it was not possible to determine the half-lives of these intermediates from the hydrolysis mixtures. This means that both alkanoyl esters were cleaved at almost identical rates, although with a slight preference of the $C_1$-alkanoyl group. As an example, the HPL-chromatograms of the incubations of compound **9b** with PLE are shown in Figure 3.

In contrast, incubation of $C_4H_9/R^2$-Di*PP*ro-NDPs **10a−c**, **8b** with PLE initially showed high amounts of the $C_4H_9$-intermediate (Figure 4, e.g., the 1 min HPLC chromatogram). In addition, as for the $C_7H_{15}$ and $C_{11}H_{23}$ intermediate, the $C_9H_{19}$-chain bearing intermediate **2′b** was also detected in the HPL-chromatograms although to a minor extent. In contrast to the above study with the $CH_3$-bearing Di*PP*ro compounds, here obviously the longer lipophilic chain acyl groups are cleaved faster than the $C_4H_9$-alkanoyl ester. This can be concluded from the markedly different amounts of the two possible intermediates in the HPL-chromatograms (Figure 4). From both formed intermediates AZTDP was finally released with a half-life of about 19 min as the sole hydrolysis product.

These observations contrasted also with the results obtained in CEM/0 cell extracts and point to a different substrate

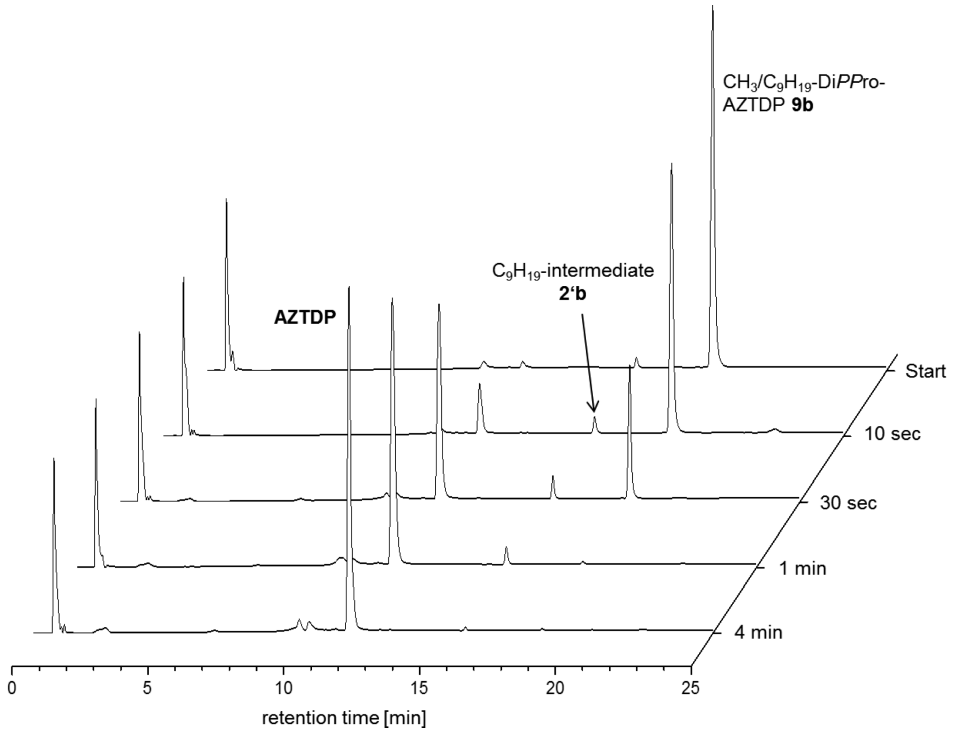


**Figure 3.** RP-18-HPLC chromatograms of the PLE-mediated hydrolysis of $CH_3/C_9H_{19}$-Di*PP*ro-AZTDP **9b**.



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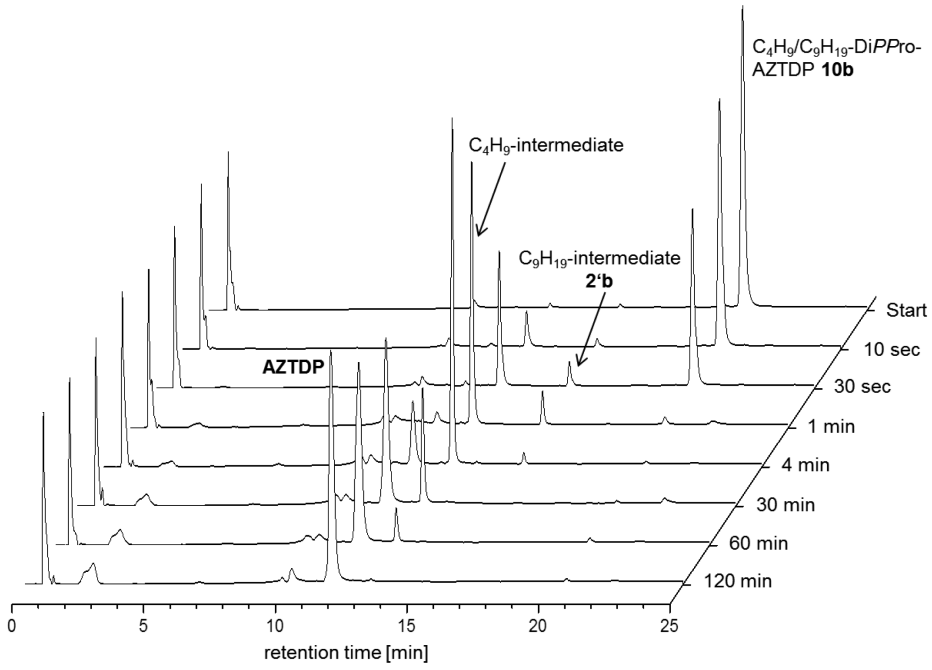


**Figure 4.** RP-18-HPLC chromatograms of the PLE-mediated hydrolysis of $C_4H_9/C_9H_{19}$-Di*PP*ro-AZTDP **10b**.

specificity of the esterase from pig liver and the esterases present in human CEM/0 cells or other enzymes that play a significant role in the hydrolysis process of the Di*PP*ro compounds described here.

*Comparative Hydrolysis Studies.* Finally, we compared the nonsymmetric Di*PP*ro-nucleotides described here with compounds **11** bearing identical masking groups. Therefore, nonsymmetric and the symmetric counterparts Di*PP*ro-AZTDP derivatives with R = $C_4H_9$ (**11a**), $C_6H_{13}$ (**11b**), and $C_9H_{19}$ (**11c**) were incubated in phosphate buffer at pH 7.3 and in CEM/0 cell extracts. The hydrolysis studies in PBS led to significant differences in the ratios of formed NDP and NMP. In all cases the symmetric compounds **11** formed markedly more NMP and less NDP as compared to the studies of the nonsymmetric Di*PP*ro compounds disclosed here. Even more interesting was the comparison of the hydrolysis in CEM/0 cell extracts. Also under these conditions the hydrolysis of the symmetric $C_4H_9$-Di*PP*ro-AZTDP **11a** and $C_6H_9$-Di*PP*ro-AZTDP **11b** resulted in high NDP/NMP ratios. In contrast, this ratio decreased significantly in the case of the more lipophilic $C_9H_{19}$-Di*PP*ro-AZTDP **11c** which was correlated to the difference in the rate of metabolism. As mentioned above, the short chain alkyl esters are rapidly cleaved by the esterases present in the extracts, and therefore, the side reaction did not interfere as in the case of the more stable long chain aliphatic esters. Figure 5 summarizes a comparison of the hydrolysis of $CH_3/C_9H_{19}$-Di*PP*ro-AZTDP **9b**, $C_9H_{19}$-Di*PP*ro-AZTDP **11c**, and the nucleoside diphosphate AZTDP after 10 h of incubation. The analysis of the hydrolysis aliquots was performed by means of HILIC-HPLC. The NDP/NMP ratio in the case of the nonsymmetric prodrug **9b** was found to be 5:1. For the symmetric prodrug **11c** the ratio was only 1.5:1. Interestingly, starting from independently synthesized AZTDP, the ratio was found to be 3:1 after 10 h of incubation in CEM/0 cell extracts.

The markedly higher ratio found in the case of the nonsymmetric Di*PP*ro-prodrug **9b** compared to AZTDP implies that compound **9b** is not susceptible to cleavage at

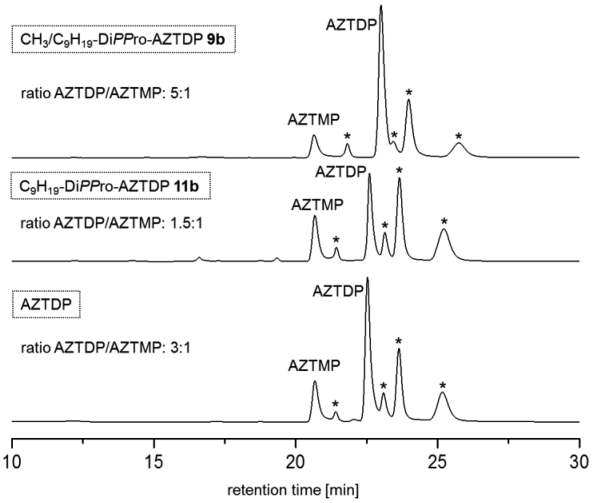


**Figure 5.** HPL-chromatograms (HILIC column) of the incubations of $CH_3/C_9H_{19}$-Di*PP*ro-AZTDP **9b**, $C_9H_{19}$-Di*PP*ro-AZTDP **11c**, and AZTDP in CEM/0 cell extracts after 10 h. The asterisk (∗) indicates components from the cell extracts.

the pyrophosphate moiety. The formation of the NMP only took place by the enzymatic dephosphorylation *after* AZTDP delivery and thus after cleavage of the masking units. As a consequence, the NDP/NMP ratio in the case of the prodrug **9b** was even higher as compared to that of AZTDP for which the dephosphorylation immediately starts at the beginning of the incubation. On the other hand, because of the high stability of the symmetric Di*PP*ro compound **11c** against enzymatic cleavage of the prodrug moieties, the chemical cleavage of the diphosphate unit in this compound occurred by the above-described concurrence reaction which led to AZTMP and consequently to a low NDP/NMP ratio (1.5:1).

**Antiviral Activity.** All Di*PP*ro compounds **7**−**11** were evaluated for their anti-HIV activity in HIV-1- and HIV-2-infected wild-type CEM/0 cell cultures and in HIV-2-infected


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Table 4. Anti-HIV and Cytostatic Activity of the Di*PP*ro-NDPs 7−11

| | $EC_{50}$[a] [μM] | | | $CC_{50}$[b] [μM] |
|---|---|---|---|---|
| compd | CEM/0 HIV-1 (HE) | CEM/0 HIV-2 (ROD) | CEM/TK$^-$ HIV-2 (ROD) | CEM/0 |
| $CH_3/C_7H_{15}$-Di*PP*ro-d4TDP **7a** | 0.33 ± 0.11 | 1.05 ± 0.30 | 6.31 ± 0.00 | 33 ± 27 |
| $CH_3/C_9H_{19}$-Di*PP*ro-d4TDP **7b** | 0.33 ± 0.11 | 1.36 ± 0.13 | 2.32 ± 1.6 | 15 ± 1 |
| $CH_3/C_{11}H_{23}$-Di*PP*ro-d4TDP **7c** | 0.29 ± 0.16 | 0.28 ± 0.04 | 1.77 ± 0.33 | 26 ± 0 |
| $CH_3/CF_3$-Ph-Di*PP*ro-d4TDP **7d** | 0.72 ± 0.16 | 1.38 ± 0.16 | >10 | 51 ± 15 |
| $CH_3/CH_3$-Ph-Di*PP*ro-d4TDP **7e** | 0.29 ± 0.057 | 1.47 ± 0.29 | 10 ± 0.00 | 11 ± 6 |
| $C_4H_9/C_7H_{15}$-Di*PP*ro-d4TDP **8a** | 0.33 ± 0.11 | 1.47 ± 0.29 | 6.31 ± 0.00 | 66 ± 15 |
| $C_4H_9/C_9H_{19}$-Di*PP*ro-d4TDP **8b** | 0.36 ± 0.046 | 1.17 ± 0.40 | 0.83 ± 0.00 | 20 ± 1 |
| $C_4H_9/C_{11}H_{23}$-Di*PP*ro-d4TDP **8c** | 0.10 ± 0.03 | 0.28 ± 0.04 | 0.13 ± 0.07 | 30 ± 17 |
| $C_4H_9/CF_3$-Ph-Di*PP*ro-d4TDP **8d** | 0.40 ± 0.00 | 0.72 ± 0.16 | >10 | 37 ± 31 |
| $C_4H_9/CH_3$-Ph-Di*PP*ro-d4TDP **8e** | 0.3 ± 0.014 | 1.45 ± 0.00 | 10 ± 0.00 | 14 ± 1 |
| $CH_3/C_7H_{15}$-Di*PP*ro-AZTDP **9a** | 0.042 ± 0.008 | 0.16 ± 0.08 | >10 | 29 ± 3 |
| $CH_3/C_9H_{19}$-Di*PP*ro-AZTDP **9b** | 0.016 ± 0.0 | 0.14 ± 0.08 | >10 | 20 ± 9 |
| $CH_3/C_{11}H_{23}$-Di*PP*ro-AZTDP **9c** | 0.042 ± 0.008 | 0.08 ± 0.00 | 10 ± 2.61 | 18 ± 3 |
| $CH_3/CF_3$-Ph-Di*PP*ro-AZTDP **9d** | 0.012 ± 0.007 | 0.06 ± 0.03 | >10 | 100 ± 8 |
| $C_4H_9/C_7H_{15}$-Di*PP*ro-AZTDP **10a** | 0.036 ± 0.0 | 0.07 ± 0.01 | >10 | 73 ± 12 |
| $C_4H_9/C_9H_{19}$-Di*PP*ro-AZTDP **10b** | 0.034 ± 0.018 | 0.29 ± 0.18 | 10 ± 0.00 | 41 ± 23 |
| $C_4H_9/C_{11}H_{23}$-Di*PP*ro-AZTDP **10c** | 0.013 ± 0.005 | 0.06 ± 0.02 | 8.16 ± 2.61 | 36 ± 28 |
| $C_4H_9/CF_3$-Ph-Di*PP*ro-AZTDP **10d** | 0.036 ± 0.0 | 0.06 ± 0.02 | >10 | 58 ± 54 |
| $C_4H_9$-Di*PP*ro-AZTDP **11a** | 0.011 ± 0.0018 | 0.054 ± 0.01 | >10 | 60 ± 7 |
| $C_6H_{13}$-Di*PP*ro-AZTDP **11b** | 0.016 ± 0.0 | 0.058 ± 0.03 | >10 | 47 ± 8 |
| $C_9H_{19}$-Di*PP*ro-AZTDP **11c** | 0.026 ± 0.014 | 0.23 ± 0.15 | 1.3 ± 0.0 | 38 ± 10 |
| d4T | 0.52 ± 0.32 | 2.23 ± 1.51 | 150 ± 9 | 79 ± 3 |
| AZT | 0.044 ± 0.01 | 0.49 ± 0.57 | >250 | >250 |

[a]Antiviral activity in CEM T-lymphocyte cultures: 50% effective concentration. [b]Cytostatic activity: 50% cytostatic concentration to inhibit CEM/0 cell proliferation by 50%.

mutant thymidine kinase-deficient CEM/TK$^-$ cell cultures. Table 4 summarizes the antiviral and cytostatic data of the Di*PP*ro compounds **7**−**10** and the parent nucleoside analogues d4T and AZT, respectively, as reference compounds.

D4TDP derivatives **7**, **8** bearing aliphatic ester functions in the acyloxybenzyl units (**a**−**c**) proved to be antivirally active against HIV-1 and HIV-2 at least in the same concentration range as compared to the parent compound d4T in wild-type CEM/0 cell cultures. Often the inhibitory potency was even somewhat higher as compared to that of the parent nucleoside particularly for compound **8c**. The antiviral activity observed in the wild-type CEM/0 cell cultures was completely retained in the case of the lipophilic Di*PP*ro compounds **7b** and **8b**,**c** in mutant thymidine-deficient CEM cells (TK$^-$). Moreover, the antiviral activity increased with the increase of lipophilicity associated with the introduced acyl moieties of the Di*PP*ro prodrugs.

Disappointingly, Di*PP*ro-d4TDPs **7**, **8** bearing benzoyl ester functions in the masking units (**d**, **e**) proved to be less potent as compared to the alkanoyl counterparts. They also were less active as compared to d4T in the wild-type CEM/0 cell cultures and lost more or less all antiviral activity in the TK-deficient CEM cell cultures ($EC_{50} \geq 10$ μM).

Like the d4TDP-containing Di*PP*ro compounds, the AZTDP derivatives **9** and **10** were able to inhibit the HIV-1 and HIV-2 induced cytopathogenic effect at very low concentrations in wild-type CEM/0 cell cultures.

In mutant thymidine kinase-deficient CEM/TK$^-$ cell cultures only the lipophilic compounds **9c**, **10b**, and **10c** showed some antiviral activity ($EC_{50} \approx 10$ μM). Interestingly, the symmetric $C_9H_{19}$-Di*PP*ro compound **11c** was active at 1.3 μM in the TK-deficient CEM cell cultures. One reason for this pronounced retention of antiviral activity of **11c** might be that the lipophilicity of this compound was sufficiently high to allow a passage through the cell membrane. In the hydrolysis studies discussed above it was shown that all compounds bearing an acetyl ester as an acyl moiety in the masking group were cleaved extremely fast. Thus, if these compounds are not lipophilic enough, they will be cleaved in the cell culture medium before the uptake into the cells. Then AZTDP will be formed extracellularly which will be enzymatically dephosphorylated to give AZT. AZT is able to migrate through the membrane, and this results in antiviral activity in the wild-type cell cultures. It has been reported that the phosphorylation of AZTMP to AZTDP by dTMP kinase is rate-limiting.[15,16] However, another reason for the failure of the Di*PP*ro-AZTDP compounds might be that even the delivered AZTDP is not efficiently metabolized into the corresponding triphosphate. In such a case the delivery of AZTDP will not lead to increased antiviral activity. It was already reported that in biochemical activity studies using nucleoside diphosphate kinase (NDP-K), which is generally the enzyme that is involved in the phosphorylation of nucleoside diphosphates into their triphosphate forms, the phosphorylation to AZTTP was rather poor.[27] So maybe there is not only a bottleneck in the metabolism of AZTMP to AZTDP[28] but also a bottleneck in the conversion of AZTDP to form AZTTP which becomes more prominent if the concentration of AZTDP is increased because of its delivery from a prodrug. From our studies in CEM/0 cell extracts we clearly have proven that the exclusively formed product from the prodrug was AZTDP.



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## SUMMARY AND CONCLUSION

Nonsymmetric Di*PP*ro-NDPs **1** of the anti-HIV active nucleoside analogues d4T and AZT are disclosed here bearing two different masking units attached to the $\beta$-phosphate group of the corresponding nucleoside diphosphate. For the synthesis of the title compounds, a new protocol for the synthesis of nonsymmetric bis(acyloxybenzyl)phosphoramidites was developed, which yielded the phosphoramidites in high chemical yields. These were coupled by weak acid catalysis with nucleoside monophosphates followed by oxidation. By use of this route, Di*PP*ro-d4TDP and Di*PP*ro-AZTDP compounds with different combinations of aliphatic and aromatic acyl moieties were obtained in good yields. Hydrolysis studies in phosphate buffer, RPMI-culture medium, CEM/0 cell extracts, and pig liver esterase confirmed the designed delivery mechanism described above. Half-lives in PBS and RPMI-culture medium were markedly higher than those in CEM/0 cell extracts and incubations with PLE. Although in PBS still both the nucleoside diphosphates and the monophosphates were formed, the exclusive products formed by enzymatic hydrolysis of the prodrugs (cell extracts and PLE) were the nucleoside diphosphates. As compared to the symmetric Di*PP*ro-NDPs with two identical masks reported previously, the ratio of formed NDP to NMP was markedly higher in all media. Most of the nonsymmetric Di*PP*ro compounds were as active as or even more active than the corresponding parent nucleosides in wild-type CEM/0 cell cultures. Moreover, also high activities were obtained depending on the lipophilicity of the Di*PP*ro-d4TDP prodrugs against HIV-2 in mutant CEM/TK$^-$ cell cultures. This confirmed that these compounds were efficiently taken up by the cells and delivered intracellularly a phosphorylated form of d4T, most likely d4T diphosphate. Surprisingly, the good antiviral activities of Di*PP*ro-AZTDPs in the wild-type cell cultures were only weakly retained in the mutant TK-deficient cell cultures. This may point to a possible further bottleneck in the phosphorylation of the AZTDP to AZTTP. A clear conclusion from the antiviral activity data was that Di*PP*ro compounds bearing short acetyl ester or benzoyl ester groups in one of the prodrug moieties were not suitable for further development. The first ester group is cleaved too fast and is not lipophilic enough to allow a successful uptake into the target cells, while the second type of ester moiety is also not lipophilic enough although we have shown before that the stability of such compounds could be adjusted over a wide range.[26] Nevertheless, this report on nonsymmetric Di*PP*ro compounds proved the successful and selective nucleoside diphosphate delivery as a further development of the Di*PP*ro-approach.

## EXPERIMENTAL SECTION

**General.** All experiments involving water-sensitive compounds were conducted under anhydride conditions and nitrogen atmosphere. All solvents were dried over an appropriate drying agent. Triethylamine and acetonitrile were dried by heating under reflux over calcium hydride for several days followed by distillation. Acetonitrile was stored over 3 Å molecular sieves. THF was dried by heating under reflux over potassium followed by distillation. Petroleum ether 50−70 for chromatography was distilled before use. The evaporation of solvents was carried out on a rotary evaporator under reduced pressure or using a high-vacuum pump. Preparative radial chromatography was performed by chromatotron (Harrison Research, model 7924T) with glass plates coated with 1, 2, or 4 mm layers of VWR 60 $PF_{254}$ silica gel containing a fluorescent indicator (VWR no. 7749). Analytical thin-layer chromatography was performed on precoated aluminum plates with a 0.2 mm layer of silica gel containing a fluorescent indicator (Macherey & Nagel Xtra Sil $UV_{254}$); sugar-containing compounds were visualized by wetting the plates with 10% sulfuric acid in water and heating with a fan.

**Instrumentation.** $^1$H NMR spectroscopy was carried out using a Bruker AMX 400 or AV 400 at 400 MHz or a Bruker AV 600 at 600 MHz with $CDCl_3$ or MeOD-$d_4$ as internal standards. $^{13}$C NMR spectra were recorded on a Bruker AMX 400 or AV 400 at 101 MHz or on a Bruker AV 600 at 150 MHz ($CDCl_3$ or MeOD-$d_4$ as internal standard). $^{31}$P NMR spectra were recorded on a Bruker AMX 400 at 162 MHz or a Bruker AV 600 at 243 MHz ($H_3PO_4$ as internal standard). $^{19}$F NMR spectra were recorded on a Bruker AV 600 MHz. All $^1$H, $^{31}$P, and $^{13}$C NMR chemical shifts are quoted in parts per million (ppm). All $^{13}$C and $^{31}$P NMR spectra were recorded in the proton-decoupled mode. High resolution ESI mass spectra were recorded with an Agilent 6224 ESI-TOF spectrometer in positive or negative modes. MALDI mass spectra were recorded on an ultrafleXtreme MALDI-TOF-TOF mass spectrometer by Bruker Daltonik with 9-AA as matrix. All HPLC measurements were carried out using a VWR Hitachi "Elite LaChrom" (pump L-2130, autosampler L-2200, column oven L-2300, diode array detector L-2455). Analytical Rp18-HPLC: Macherey & Nagel EC 125/3 NUCLEODUR 100-5 C18 EC. As eluents $CH_3CN$/$[(C_4H_9)_4N]^+CH_3COO^-$ buffer (2 mM, pH 6.0) gradient was used. Method A: 0−24 min $CH_3CN$ (5−80%), 24−29 min $CH_3CN$ (80%), flow rate 1 mL/min. Method B: 0−27 min $CH_3CN$ (5−90%), 27−30 min $CH_3CN$ (90%), flow rate 1 mL/min, column temperature of 25 °C. UV detection at 265 nm. The purities of the Di*PP*ro nucleotides were evaluated by means of HPLC and were in all cases ≥95%. Analytical HILIC-HPLC: Macherey & Nagel EC 125/4 NUCLEODUR HILIC, 5 $\mu$m. As eluents, $CH_3CN/NH_4^+HCOO^-$ buffer (10 mM, pH 8.3) gradient was used. Method C: 0−5 min $CH_3CN$ (95%), 5−15 min $CH_3CN$ (95−70%), 15−30 min $CH_3CN$ (70%), flow rate 0.6 mL/min, column temperature of 25 °C. UV detection at 265 nm.

**Synthesis. General Procedure A. Preparation of Acyloxybenzyl Bis(diisopropylamino)phosphoramidite 6.** To a low concentrated solution of 1 equiv of phosphorus trichloride and 1 equiv of pyridine in THF, 1 equiv of 4-acyloxybenzyl alcohol dissolved in THF was added dropwise over 1.5 h at −78 °C. After removal of the cold bath the reaction was stirred for about 20 h at room temperature until the total reaction of 4-acyloxybenzyl alcohol was accomplished. Then an amount of 6.1 equiv of *N*,*N*-diisopropylamine was added dropwise at −10 °C. After removal of the cold bath the reaction was stirred for about 1−2 days at room temperature. Salts were removed by filtration. The crude product was purified by radial chromatography at the chromatotron with petroleum ether/triethylamine as eluent.

**General Procedure B. Preparation of Nonsymmetric Bis(acyloxybenzyl)phosphoramidites 5.** To a solution of 1.5 equiv of phosphordiamidite in acetonitrile, 1 equiv of 4-acyloxybenzyl alcohol and 1 equiv of a DCI-activator solution (0.25 M in acetonitrile) also dissolved in acetonitrile were added dropwise at 4 °C. After removal of the cold bath the reaction mixture was stirred for 30−60 min. The reaction was stopped by removing the solvent under reduced pressure. The residue was dissolved in petroleum ether/triethylamine (9:1) and filtered. The crude product was purified by radial chromatography at the chromatotron with petroleum ether/triethylamine as eluent.

**General Procedure C. Preparation of Di*PP*ro-Nucleoside Diphosphates.** An amount of 1.5 equiv of phosphoramidite **5** was added to a solution of 1 equiv of the bis(tetra-*n*-butylammonium) nucleoside monophosphate in acetonitrile. The reaction was started by addition of 0.5 equiv of 4,5-dicyanoimidazole (0.25 M solution in acetonitrile) at room temperature. Every 5 min again 0.25 equiv of 4,5-dicyanoimidazole was added up to a total of 1.25−1.75 equiv. After stirring another 5 min after the last addition, the mixture was oxidized by addition of 1.5 equiv of *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane). The solvents were removed under reduced pressure after 15 min. The compounds were isolated by automatic flash RP18-chromatography (acetonitrile/water). After the tetra-*n*-butylammonium ions were changed with ammonia by elution over DOWEX


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50WX8 ($NH_4^+$), a second automatic flash RP18 chromatography (acetonitrile/water) followed.

Syntheses of 4-(hydroxymethyl)phenylacetate **3a**,[24] 4-(hydroxymethyl)phenylpentanoate **3b**,[25] 4-(hydroxymethyl)-phenyloctanoate **4a**,[24] 4-(hydroxymethyl)phenyldecanoate **4b**,[25] 4-(hydroxylmethyl)phenyldodecanoate **4c**,[25] 4-(hydroxymethyl)phenyl-4′-trifluoromethylbenzoate **4d**,[26] 4-(hydroxymethyl)phenyl-4′-methyl-benzoate **4e**,[26] and 4-(hydroxymethyl)phenylheptanoate **4f**[25] have been described before. The analytical data were identical to those reported before.

Syntheses of $(N[n\text{-}Bu]_4)_2$-d4TMP and $(N[n\text{-}Bu]_4)_2$-AZTMP have been described before.[24,25] The analytical data were identical to those reported before.

**Acetoxybenzyl Bis(diisopropylamino)phosphoramidite 6a.** General procedure B; 4-acyloxybenzyl alcohol **3a** (2.36 g, 14.4 mmol), dissolved in 15 mL of THF and phosphorus trichloride (1.26 mL, 14.4 mmol) and pyridine (1.17 mL, 14.4 mmol) in 35 mL of THF. In the following step DIPA (12.4 mL, 87.8 mmol) was added. The product (3.02 g, 7.62 mmol, 53%) was obtained as a colorless waxlike solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ [ppm] = 7.39−7.37 (m, 2 H, H-2); 7.05−7.04 (m, 2 H, H-3); 4.64−4.63 (m, 2 H, Ph-$CH_2$); 3.61−3.54 (m, 4 H, CN-H); 2.29 (s, 3 H, H-6); 1.19 (d, $^3J_{H,H}$ = 6.9 Hz, 12 H, *iso*Pr); 1.19 (d, $^3J_{H,H}$ = 7.0 Hz, 12 H, *iso*Pr). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ [ppm] = 169.6 (C-5); 149.6 (C-4); 138.3 (d, $^3J_{C,P}$ = 10.6 Hz, C-1); 127.9 (C-2); 121.3 (C-3); 65.6 (d, $^2J_{C,P}$ = 23.2 Hz, Ph-$CH_2$); 44.5 (d, $^1J_{C,N}$ = 12.3 Hz, 2 × N-CH); 24.7 (d, $^2J_{C,N}$ = 8.0 Hz, *iso*Pr); 24.0 (d, $^2J_{C,N}$ = 5.6 Hz, *iso*Pr); 21.2 (C-6). $^{31}$P NMR (162 MHz, $CDCl_3$, decoupled): $\delta$/ppm =123.4; IR $\tilde{\nu}$ [$cm^{-1}$] = 2970, 2923, 2901, 1766, 1506, 1454, 1302, 1114, 1049, 950, 741. MS (MALDI) *m*/*z* = calcd for $C_{34}H_{46}N_3O_6P$: 589.331 [M − $H^+$ + 9-aminoacridine (9-AA) used as matrix]$^-$. Found: 589.309.

**Pentanoyloxybenzyl Bis(diisopropylamino)-phosphoramidite 6b.** General procedure B; 4-acyloxybenzyl alcohol **3b** (2.25 g, 10.8 mmol), dissolved in 15 mL of THF and phosphorus trichloride (0.94 mL, 10.8 mmol) and pyridine (0.88 mL, 10.8 mmol) in 35 mL of THF. In the following step DIPA (9.24 mL, 65.8 mmol) was added. The product (2.65 g, 6.04 mmol, 56%) was obtained as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ [ppm] = 7.38−7.36 (m, 2 H, H-2); 7.04−7.02 (m, 2 H, H-3); 4.64−4.62 (m, 2 H, Ph-$CH_2$); 3.62−3.52 (m, 4 H, CN-H); 2.29 (t, $^3J_{HH}$ = 7.5 Hz, 2 H, H-6); 1.78−1.70 (m, 2H, H-7); 1.50−1.40 (m, 2H, H-8); 1.18 (d, $^3J_{H,H}$ = 6.8 Hz, 12 H, *iso*Pr); 1.18 (d, $^3J_{H,H}$ = 6.9 Hz, 12 H, *iso*Pr); 0.97 (t, $^3J_{H,H}$ = 7.4 Hz, 2 H, H-9). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ [ppm] = 172.5 (C-5); 149.7 (C-4); 138.2 (d, $^3J_{C,P}$ = 10.7 Hz, C-1); 127.9 (C-2); 121.3 (C-3); 65.7 (d, $^2J_{C,P}$ = 23.0 Hz, Ph-$CH_2$); 44.6 (d, $^1J_{C,N}$ = 12.4 Hz, 2 × N-CH); 34.3 (C-6); 27.2 (C-7); 24.8 (d, $^2J_{C,N}$ = 7.9 Hz, *iso*Pr); 24.0 (d, $^2J_{C,N}$ = 5.7 Hz, *iso*Pr); 22.4 (C-8); 13.9 (C-9). $^{31}$P NMR (162 MHz, $CDCl_3$, decoupled): $\delta$/ppm =123.5. IR $\tilde{\nu}$ [$cm^{-1}$] = 2964, 2930, 2870, 1760, 1507, 1390, 1195, 1182, 1144, 1016, 951, 779. MS (MALDI) *m*/*z* = calcd for $C_{37}H_{52}N_3O_6P$: 631.386 [M + 9-aminoacridine (9-AA) used as matrix]. Found: 631.368.

**$CH_3$/$C_7H_{15}$-phosphoramidite 5a.** General procedure C; acetoxybenzylbis(diisopropylamino)phosphoramidite **6a** (1.24 g, 3.12 mmol) dissolved in 15 mL of acetonitrile and 4-(hydroxymethyl)-phenyloctanoate **4a** (520 mg, 2.08 mmol) and DCI-activator solution (8.32 mL, 2.08 mmol) dissolved in 5 mL of acetonitrile. The product (932 mg, 1.71 mmol, 82%) was obtained as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ [ppm] = 7.36−7.34 (m 4H, H-2, H-2′); 7.05−7.03 (m, 4H, H-3, H-3′); 4.77−4.73 (m, 2H, Ph-$CH_2$); 4.70−4.66 (m, 2H, Ph-$CH_2$); 3.72−3.66 (m, 2H, 2 × NC-H); 2.56−2.53 (m, 2H, H-6′); 2.29 (s, 3H, −$CH_3$); 1.78−1.73 (m, 2H, H-7′); 1.44−1.29 (m, 12H, H-8′−H-11′); 1.20 (d, $^3J_{H,H}$ = 6.8 Hz, 12H, *iso*Pr); 0.90 (d, $^3J_{H,H}$ = 6.8 Hz, 3H, H-12′). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ [ppm] = 172.5 (C-5′); 169.6 (C-5); 150.1 (C-4′); 150.0 (C-4); 137.3 (d, $^3J_{C,P}$ = 7.6 Hz, C-1′); 137.1 (d, $^3J_{C,P}$ = 7.5 Hz, C-1); 128.1, 128.1 (C-2, C-2′); 121.5, 121.5 (C-3, C-3′); 65.0 (d, $^2J_{C,P}$ = 4.7 Hz, Ph-$CH_2$); 64.9 (d, $^2J_{C,P}$ = 4.8 Hz, Ph-$CH_2$); 43.3 (d, $^1J_{C,N}$ = 12.5 Hz, N-CH); 34.6 (C-6′); 31.8, 29.2, 29.6, 22.7, (C-8′−C-11′); 25.1 (C-7′); 24.8, 24.8 (2 × *iso*Pr); 21.3 (C-6); 14.2 (C-12′). $^{31}$P NMR (162 MHz, $CDCl_3$, decoupled): $\delta$/ppm =148.0. IR $\tilde{\nu}$ [$cm^{-1}$] = 2965, 2929, 2859, 1760, 1608, 1507, 1366, 1192, 1163, 1005, 973, 756, 504. HRMS ($ESI^+$) *m*/*z* = calcd for $C_{30}H_{45}NO_6P$: 546.2979 [M + $H^+$]$^+$. Found: 546.2951.

**$CH_3$/$C_9H_{19}$-phosphoramidite 5b.** General procedure C; acetoxybenzylbis(diisopropylamino)phosphoramidite **6a** (520 mg, 1.31 mmol) dissolved in 10 mL of acetonitrile and 4-(hydroxymethyl)-phenyldecanoate **4b** (244 mg, 0.88 mmol) and DCI-activator solution (3.52 mL, 0.88 mmol) dissolved in 6 mL of acetonitrile. The product (437 mg, 0.76 mmol, 87%) was obtained as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ [ppm] = 7.36−7.34 (m 4H, H-2, H-2′); 7.05−7.02 (m, 4H, H-3, H-3′); 4.77−4.64 (m, 4H, 2 × Ph-$CH_2$); 3.73−3.64 (m, 2H, 2 × NC-H); 2.56−2.52 (m, 2H, H-6′); 2.29 (s, 3H, −$CH_3$); 1.79−1.71 (m, 2H, H-7′); 1.44−1.27 (m, 12H, H-8′−H-13′); 1.20 (d, $^3J_{H,H}$ = 6.8 Hz, 12H, *iso*Pr); 0.89 (d, $^3J_{H,H}$ = 6.8 Hz, 3H, H-14′). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ [ppm] = 172.5 (C-5′); 169.6 (C-5); 150.2 (C-4′); 150.0 (C-4); 137.2 (d, $^3J_{C,P}$ = 7.4 Hz, C-1, C-1′); 128.2, 128.1 (C-2, C-2′); 121.5, 121.5 (C-3, C-3′); 65.1 (d, $^2J_{C,P}$ = 3.2 Hz, Ph-$CH_2$); 64.9 (d, $^2J_{C,P}$ = 3.4 Hz, Ph-$CH_2$); 43.3 (d, $^1J_{C,N}$ = 12.4 Hz, N-CH); 34.6 (C-6′); 32.0, 29.6, 29.4, 29.3, 22.8, (C-8′−C-13′); 25.1 (C-7′); 24.8, 24.8 (2 × *iso*Pr); 21.3 (C-6); 14.2 (C-14′). $^{31}$P NMR (162 MHz, $CDCl_3$, decoupled): $\delta$/ppm =148.0. IR $\tilde{\nu}$ [$cm^{-1}$] = 2964, 2926, 2855, 1759, 1507, 1192, 1005, 972, 755, 503. HRMS ($ESI^+$) *m*/*z* = calcd for $C_{32}H_{49}NO_6P$: 574.3292 [M + $H^+$]$^+$. Found: 574.3196.

**$CH_3$/$C_{11}H_{23}$-phosphoramidite 5c.** General procedure C; acetoxybenzylbis(diisopropylamino)phosphoramidite **6a** (620 mg, 1.57 mmol) dissolved in 12 mL of acetonitrile and 4-(hydroxymethyl)-phenyldodecanoate **4c** (319 mg, 1.04 mmol) and DCI-activator solution (4.16 mL, 1.04 mmol) in 5 mL of acetonitrile. The product (453 mg, 0.75 mmol, 72%) was obtained as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ [ppm] = 7.36−7.33 (m 4H, H-2, H-2′); 7.05−7.02 (m, 4H, H-3, H-3′); 4.78−4.64 (m, 4H, 2 × Ph-$CH_2$); 3.73−3.64 (m, 2H, 2 × NC-H), 2.56−2.52 (m, 2H, H-6′); 2.29 (s, 3H, -$CH_3$); 1.78−1.71 (m, 2H, H-7′); 1.43−1.26 (m, 16H, H-8′−H-15′); 1.20 (d, $^3J_{H,H}$ = 6.8 Hz, 12H, *iso*Pr); 0.89 (d, $^3J_{H,H}$ = 6.8 Hz, 3H, H-16′). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ [ppm] = 172.5 (C-5′); 169.5 (C-5); 150.2 (C-4′); 150.0 (C-4); 137.2 (C-1, C-1′); 128.2 (C-2, C-2′); 121.5 (C-3, C-3′); 65.0 (d, $^2J_{C,P}$ = 18.4 Hz, Ph-$CH_2$); 65.0 (d, $^2J_{C,P}$ = 18.9 Hz, Ph-$CH_2$); 43.3 (d, $^1J_{C,N}$ = 12.2 Hz, N-CH); 34.6 (C-6′); 29.8, 29.7, 29.6, 29.5, 29.4, 29.4, 29.3 (C-8′−C-15′); 25.1 (C-7′); 24.8, 24.8 (2 × *iso*Pr); 21.3 (C-6); 14.4 (C-16′). $^{31}$P NMR (162 MHz, $CDCl_3$, decoupled): $\delta$/ppm =148.0. IR $\tilde{\nu}$ [$cm^{-1}$] = 2964, 2925, 2854, 1760, 1608, 1507, 1365, 1192, 1163, 1005, 973, 756, 503. HRMS ($ESI^+$) *m*/*z* = calcd for $C_{34}H_{53}NO_6P$: 602.3605 [M + $H^+$]$^+$. Found: 602.4100.

**$CH_3$/$CF_3$-Ph-phosphoramidite 5d.** General procedure C; acetoxybenzylbis(diisopropylamino)phosphoramidite **6a** (544 mg, 1.37 mmol) dissolved in 12 mL of acetonitrile and 4-(hydroxymethyl)-phenyl-4′-trifluoromethylbenzoate **4d** (271 g, 0.915 mmol) and DCI-activator solution (3.66 mL, 0.915 mmol) in 5 mL of acetonitrile. The product (360 mg, 0.61 mmol, 67%) was obtained as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ [ppm] = 8.33−8.32 (m 2H, H-7′); 7.79−7.78 (m, 2H, H-8′); 7.43−7.41 (m, 2H, H-2′); 7.37−7.36 (m, 2H, H-2); 7.19−7.18 (m, 2H, H-3′); 7.06−7.05 (m, 2H, H-3); 4.81−4.67 (m, 4H, 2 × Ph-$CH_2$); 3.74−3.68 (m, 4H, CN-H); 2.29 (s, 3H, $CH_3$); 1.22 (d, $^3J_{H,H}$ = 6.7 Hz, 6H, *iso*Pr); 1.22 (d, $^3J_{H,H}$ = 6.5 Hz, 6H, *iso*Pr). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ [ppm] = 169.7 (C-5); 164.2 (C-5′); 150.0 (C-4); 149.9 (C-4); 137.7 (d, $^3J_{C,P}$ = 7.5 Hz, C-1′); 137.2 (d, $^3J_{C,P}$ = 7.8 Hz, C-1); 135.2 (d, $^3J_{C,F}$ = 33 Hz, C-9′), 133.0 (C-6′); 130.7 (C-8′); 128.3 (C-2′); 128.3 (C-2); 125.8 (d, $^3J_{C,F}$ = 3.6 Hz, C-7′); 125.7 ($CF_3$); 121.5, 121.5 (C-3, C-3′); 65.0 (d, $^2J_{C,P}$ = 18.5 Hz, Ph-$CH_2$); 65.0 (d, $^2J_{C,P}$ = 18.6 Hz, Ph-$CH_2$); 43.2 (d, $^1J_{C,N}$ = 12.3 Hz, N-CH); 24.8, 24.8 (d, $^2J_{C,N}$ = 7.3 Hz, 2 × *iso*Pr); 21.3 (C-6). $^{19}$F NMR (565 MHz, $CDCl_3$): $\delta$/ppm =67.1. $^{31}$P NMR (162 MHz, $CDCl_3$, decoupled): $\delta$/ppm =148.1. IR $\tilde{\nu}$ [$cm^{-1}$] = 2968, 2932, 2869, 1762, 1741, 1608, 1506, 1324, 1264, 1193, 1129, 1075, 1013, 770, 552. HRMS ($ESI^+$) *m*/*z* = calcd for $C_{30}H_{34}F_3NO_6P$: 592.2071 [M + $H^+$]$^+$. Found: 592.2209.

**$CH_3$/$CH_3$-Ph-phosphoramidite 5e.** General procedure C; acetoxybenzylbis(diisopropylamino)phosphoramidite **6a** (505 mg, 1.27 mmol) dissolved in 10 mL of acetonitrile and 4-(hydroxymethyl)-phenyl-4′-methylbenzoate **4e** (205 mg, 0.85 mmol) and DCI-activator solution (3.40 mL, 0.85 mmol) in 5 mL of acetonitrile. The product



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J. Med. Chem. 2015, 58, 6114−6130

(323 mg, 0.60 mmol, 71%) was obtained as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ [ppm] = 8.11−8.09 (m 2H, H-7′/H-8′); 7.42−7.36 (m, 4H, H-2, H-2′); 7.32−7.30 (m, 2H, H-7′/H-8′); 7.19−7.17 (m, 2H, H-3′); 7.07−7.05 (m, 2H, H-3); 4.81- 4.67 (m, 4H, 2 × Ph-$CH_2$); 3.76−3.67 (m, 4H, CN-H); 2.45 (s, 3H, -Ph-$CH_3$); 2.29 (s, 3H, −$CH_3$); 1.22 (d, $^3J_{H,H}$ = 6.8 Hz, 6H, *iso*Pr); 1.22 (d, $^3J_{H,H}$ = 6.8 Hz, 6H, *iso*Pr). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ [ppm] = 169.6 (C-5); 165.4 (C-5′); 150.3 (C-4); 150.0 (C-4′); 144.5 (C-9′); 137.3 (d, $^2J_{C,P}$ = 8.0 Hz, C-1/C-1′); 137.2 (d, $^2J_{C,P}$ = 7.8 Hz, C-1/C-1′); 130.3 (C-7′); 129.4 (C-8′); 128.2, 128.2 (C-2/C-2′); 127.0 (C-6′); 121.7, 121.5 (C-3, C-3′); 65.0 (d, $^2J_{C,P}$ = 18.4 Hz, Ph-$CH_2$); 65.1 (d, $^2J_{C,P}$ = 18.3 Hz, Ph-$CH_2$); 43.3 (d, $^1J_{C,N}$ = 12.4 Hz, N-CH); 24.8, 24.8 (d, $^2J_{C,N}$ = 7.2 Hz, 2 × *iso*Pr); 21.9 (Ph-$CH_3$); 21.3 (C-6). $^{31}$P NMR (162 MHz, $CDCl_3$, decoupled): $\delta$/ppm =147.9. IR $\tilde{\nu}$ [$cm^{-1}$] = 2966, 2930, 2868, 1762, 1735, 1610, 1507, 1366, 1192, 1177, 1003, 972, 783, 505. HRMS (ESI$^+$) $m/z$ = calcd for $C_{30}H_{37}NO_6P$: 538.2553 [M + $H^+$]$^+$. Found: 538.2359.

**$C_4H_9$/$C_7H_{15}$-phosphoramidite 5f.** General procedure C; pentanoyloxybenzylbis(diisopropylamino)phosphoramidite **6b** (350 mg, 0.798 mmol) dissolved in 8 mL of acetonitrile and 4-(hydroxymethyl)phenyloctanoate **4a** (133 mg, 0.532 mmol) and DCI-activator solution (2.13 mL, 0.532 mmol) in 4 mL of acetonitrile. The product (281 mg, 0.478 mmol, 90%) was obtained as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ [ppm] = 7.35−7.34 (m 4H, H-2, H-2′); 7.04−7.02 (m, 4H, H-3, H-3′); 4.76−4.73 (m, 2H, Ph-$CH_2$); 4.69−4.65 (m, 2H, Ph-$CH_2$); 3.72−3.65 (m, 2H, 2 × NC-H), 2.56 (t, $^3J_{H,H}$ = 6.4 Hz, 2H, H-6′); 2.54 (t, $^3J_{H,H}$ = 6.4 Hz, 2H, H-6); 1.76−1.72 (m, 4H, H-7, H-7′); 1.48−1.29 (m, 10H, H-8, H-8′ − 11′); 1.20 (d, $^3J_{H,H}$ = 6.8 Hz, 12H, *iso*Pr); 0.97 (d, $^3J_{H,H}$ = 7.4 Hz, 3H, H-9); 0.89 (d, $^3J_{H,H}$ = 6.8 Hz, 3H, H-12′). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ [ppm] = 172.5 (C-5, C-5′); 150.1 (C-4, C-4′); 137.1 (d, $^3J_{C,P}$ = 7.4 Hz, C-1, C-1′); 128.2 (C-2, C-2′); 121.5 (C-3, C-3′); 65.0 (d, $^2J_{C,P}$ = 18.1 Hz, 2 × Ph-$CH_2$); 43.3 (d, $^1J_{C,N}$ = 12.4 Hz, N-CH); 34.6, 34.3 (C-6, C-6′); 31.8, 29.2, 29.1, 22.8, 22.4 (C-8, C-8′−C-11′); 27.2, 25.1 (C-7, C-7′); 24.8, 24.8 (2 × *iso*Pr); 14.2 (C-12′); 13.9 (C-9). $^{31}$P NMR (162 MHz, $CDCl_3$, decoupled): $\delta$/ppm =148.0. IR $\tilde{\nu}$ [$cm^{-1}$] = 2964, 2929, 2861, 1758, 1608, 1507, 1364, 1197, 1163, 1001, 973, 754, 504. HRMS (ESI$^+$) $m/z$ = calcd for $C_{33}H_{51}NO_6P$: 588.3449 [M + $H^+$]$^+$. Found: 588.3379.

**$C_4H_9$/$C_9H_{19}$-phosphoramidite 5g.** General procedure C; pentanoyloxybenzylbis(diisopropylamino)phosphoramidite **6b** (427 mg, 0.974 mmol) dissolved in 8 mL of acetonitrile and 4-(hydroxymethyl)phenyldecanoate **4b** (181 mg, 0.649 mmol) and DCI-activator solution (2.60 mL, 0.649 mmol) in 4 mL of acetonitrile. The product (344 mg, 0.559 mmol, 86%) was obtained as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ [ppm] = 7.35−7.34 (m 4H, H-2, H-2′); 7.04−7.02 (m, 4H, H-3, H-3′); 4.76−4.73 (m, 2H, Ph-$CH_2$); 4.69−4.65 (m, 2H, Ph-$CH_2$); 3.72−3.65 (m, 2H, 2 × NC-H), 2.56 (t, $^3J_{H,H}$ = 6.8 Hz, 2H, H-6′); 2.54 (t, $^3J_{H,H}$ = 6.8 Hz, 2H, H-6); 1.77−1.72 (m, 4H, H-7, H-7′); 1.48−1.38 (m, 4H, H-8′, H-8); 1.35−1.26 (m, 10H, H-9′−H-13′); 1.20 (d, $^3J_{H,H}$ = 6.8 Hz, 12H, *iso*Pr); 0.97 (d, $^3J_{H,H}$ = 7.4 Hz, 3H, H-9); 0.89 (d, $^3J_{H,H}$ = 6.9 Hz, 3H, H-14′). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ [ppm] = 172.5 (C-5, C-5′); 150.1 (C-4, C-4′); 137.1 (d, $^3J_{C,P}$ = 7.6 Hz, C-1, C-1′); 128.2 (C-2, C-2′); 121.5 (C-3, C-3′); 65.0 (d, $^2J_{C,P}$ = 18.1 Hz, 2 × Ph-$CH_2$); 43.3 (d, $^1J_{C,N}$ = 12.6 Hz, N-CH); 34.6, 34.3 (C-6, C-6′); 32.0, 29.6, 29.4, 22.8, (C-9′−C-13′); 29.3 (C-8/C-8′); 27.2, 25.1 (C-7, C-7′); 24.8, 24.8 (2 × *iso*Pr); 22.4 (C-8/C-8′); 14.2, 13.9 (C-9, C-14′). $^{31}$P NMR (162 MHz, $CDCl_3$, decoupled): $\delta$/ppm =148.0. IR $\tilde{\nu}$ [$cm^{-1}$] = 2962, 2927, 2856, 1758, 1507, 1364, 1198, 1138, 1104, 973, 754, 504. HRMS (ESI$^+$) $m/z$ = calcd for $C_{35}H_{55}NO_6P$: 616.3762 [M + $H^+$]$^+$. Found: 616.3753.

**$C_4H_9$/$C_{11}H_{23}$-phosphoramidite 5h.** General procedure C; pentanoyloxybenzylbis(diisopropylamino)phosphoramidite **6b** (376 mg, 1.08 mmol) dissolved in 8 mL of acetonitrile and 4-(hydroxymethyl)phenyldodecanoate **4c** (220 mg, 0.72 mmol) and DCI-activator solution (2.88 mL, 0.72 mmol) in 4 mL of acetonitrile. The product (174 mg, 0.242 mmol, 34%) was obtained as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ [ppm] = 7.35−7.34 (m 4H, H-2, H-2′); 7.04−7.02 (m, 4H, H-3, H-3′); 4.77−4.72 (m, 2H, Ph-$CH_2$); 4.69−4.65 (m, 2H, Ph-$CH_2$); 3.72−3.65 (m, 2H, 2 × NC-H), 2.57−2.53 (m, 2H, H-6, H-6′); 1.78−1.71 (m, 4H, H-7, H-7′); 1.49−1.26 (m, 18H, H-8, H-8′−H-15′); 1.20 (d, $^3J_{H,H}$ = 6.8 Hz, 12H, *iso*Pr); 0.97 (d, $^3J_{H,H}$ = 7.4 Hz, 3H, H-9); 0.88 (d, $^3J_{H,H}$ = 6.9 Hz, 3H, H-16′). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ [ppm] = 172.5 (C-5, C-5′); 150.0 (C-4, C-4′); 136.9 (m, C-1, C-1′); 128.1 (C-2, C-2′); 121.5 (C-3, C-3′); 65.0 (d, $^2J_{C,P}$ = 18.1 Hz, 2 × Ph-$CH_2$); 43.3 (d, $^1J_{C,N}$ = 12.5 Hz, N-CH); 34.6, 34.3 (C-6, C-6′); 32.1, 29.7, 29.4, 29.3, 22.8, 22.4 (C8, C-8′−C-13′); 27.1, 25.1 (C-7, C-7′); 24.8, 24.8 (2 × *iso*Pr); 14.2 (C-14′); 13.9 (C-9). $^{31}$P NMR (162 MHz, $CDCl_3$, decoupled): $\delta$/ppm = 148.0. IR $\tilde{\nu}$ [$cm^{-1}$] = 2962, 2925, 2855, 1758, 1507, 1311, 1198, 1138, 1004, 973, 755, 503. HRMS (ESI$^+$) $m/z$ = calcd for $C_{37}H_{59}NO_6P$: 644.4075 [M + $H^+$]$^+$. Found: 644.4068.

**$C_4H_9$/$CF_3$-Ph-phosphoramidite 5i.** General procedure C; pentanoyloxybenzylbis(diisopropylamino)phosphoramidite **6b** (717 mg, 1.64 mmol) dissolved in 12 mL of acetonitrile and 4-(hydroxymethyl)phenyl-4′-trifluoromethylbenzoate **4d** (323 mg, 1.09 mmol) and DCI-activator solution (4.36 mL, 1.09 mmol) in 5 mL of acetonitrile. The product (622 mg, 0.981 mmol, 90%) was obtained as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ [ppm] = 8.33−8.31 (m 2H, H-7′); 7.79−7.77 (m, 2H, H-8′); 7.43−7.41 (m, 2H, H-2′); 7.37−7.35 (m, 2H, H-2); 7.19−7.17 (m, 2H, H-3′); 7.05−7.03 (m, 2H, H-3); 4.81−4.66 (m, 4H, 2 × Ph-$CH_2$); 3.75- 3.66 (m, 4H, CN-H); 2.56 (t, $^3J_{H,H}$ = 7.5 Hz, 2H, H-6); 1.78−1.70 (m, 2H, H-7); 1.49−1.40 (m, 2H, H-8); 1.22 (d, $^3J_{H,H}$ = 6.8 Hz, 6H, *iso*Pr); 1.21 (d, $^3J_{H,H}$ = 6.8 Hz, 6H, *iso*Pr); 0.97 (t, $^3J_{H,H}$ = 7.3 Hz, 2H, H-9). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ [ppm] = 172.4 (C-5); 164.1 (C-5′); 150.0 (C-4); 149.9 (C-4′); 137.7 (C-1′); 137.1 (C-1); 135.0 (C-6′), 132.9 (C-9′); 130.7 (C-7′); 128.3 (C-2′); 128.2 (C-2); 125.8 (d, $^3J_{C,F}$ = 3.7 Hz, C-8′); 125.5 ($CF_3$); 121.5, 121.5 (C-3, C-3′); 65.0 (d, $^2J_{C,P}$ = 18.5 Hz, Ph-$CH_2$); 65.0 (d, $^2J_{C,P}$ = 18.3 Hz, Ph-$CH_2$); 43.2 (d, $^1J_{C,N}$ = 12.4 Hz, N-CH); 34.3 (C-6); 27.2 (C-7); 24.8, 24.8 (d, $^2J_{C,N}$ = 7.3 Hz, 2 × *iso*Pr); 22.4 (C-8); 13.9 (C-9). $^{19}$F NMR (565 MHz, $CDCl_3$): $\delta$/ppm = 63.2. $^{31}$P NMR (162 MHz, $CDCl_3$, decoupled): $\delta$/ppm = 148.1. IR $\tilde{\nu}$ [$cm^{-1}$] = 2968, 2932, 2872, 1737, 1505, 1325, 1266, 1065, 1014, 969, 712, 504. HRMS (ESI$^+$) $m/z$ = calcd for $C_{33}H_{40}F_3NO_6P$: 616.3692 [M + $H^+$]$^+$. Found: 616.3753.

**$C_4H_9$/$CH_3$-Ph-phosphoramidite 5j.** General procedure C; pentanoyloxybenzylbis(diisopropylamino)phosphoramidite **6b** (396 mg, 0.903 mmol) dissolved in 8 mL of acetonitrile and 4-(hydroxymethyl)phenyl-4′-methylbenzoate **4e** (146 mg, 0.602 mmol) and DCI-activator solution (2.4 mL, 0.60 mmol) in 4 mL of acetonitrile. The product (348 mg, 0.60 mmol, 99%) was obtained as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ [ppm] = 8.10−8.08 (m 2H, H-7′/H-8′); 7.40−7.39 (m, 2H, H-2′); 7.36−7.35 (m, 2H, H-2); 7.31−7.30 (m, 2H, H-7′/H-8′); 7.17−7.16 (m, 2H, H-3′); 7.05−7.03 (m, 2H, H-3); 4.79−4.67 (m, 4H, 2 × Ph-$CH_2$); 3.73−3.67 (m, 4H, CN-H); 2.55 (t, $^3J_{H,H}$ = 7.5 Hz, 2H, H-6); 2.45 (s, 3H, -Ph-$CH_3$); 1.76−1.71 (m, 2H, H-7); 1.48−1.41 (m, 2H, H-8); 1.21 (d, $^3J_{H,H}$ = 6.8 Hz, 6H, *iso*Pr); 1.21 (d, $^3J_{H,H}$ = 6.7 Hz, 6H, *iso*Pr); 0.97 (t, $^3J_{H,H}$ = 7.4 Hz, 2H, H-9). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ [ppm] = 172.5 (C-5); 164.4 (C-5′); 150.3 (C-4); 150.1 (C-4′); 144.5 (C-9′); 137.2 (d, $^2J_{C,P}$ = 7.6 Hz, C-1/C-1′); 137.2 (d, $^2J_{C,P}$ = 7.6 Hz, C-1/C-1′); 130.4 (C-7′); 129.4 (C-8′); 128.2, 128.2 (C-2/C-2′); 127.0 (C-6′); 121.7, 121.5 (C-3, C-3′); 65.0 (d, $^2J_{C,P}$ = 18.2 Hz, Ph-$CH_2$); 65.0 (d, $^2J_{C,P}$ = 18.1 Hz, Ph-$CH_2$); 43.2 (d, $^1J_{C,N}$ = 12.4 Hz, N-CH); 34.3 (C-6); 27.2 (C-7); 24.8, 24.8 (d, $^2J_{C,N}$ = 7.3 Hz, 2 × *iso*Pr); 22.4 (C-8); 21.9 (Ph-$CH_3$); 13.9 (C-9). $^{31}$P NMR (162 MHz, $CDCl_3$, decoupled): $\delta$/ppm = 144.0. IR $\tilde{\nu}$ [$cm^{-1}$] = 2965, 2931, 2871, 1758, 1736, 1610, 1507, 1264, 1197, 1007, 972, 756, 506. HRMS (ESI$^+$) $m/z$ = calcd for $C_{33}H_{43}NO_6P$: 616.3692 [M + $H^+$]$^+$. Found: 616.3753.

Syntheses of symmetric bis(4-pentanoyloxymethyl)-, bis(4-heptanoyloxymethyl)-, and bis(4-decanoyloxymethyl)-*N*,*N*-diisopropylaminophosphoramidite have been described before.[24,25] The analytical data were identical.

**Ammonium-$CH_3$/$C_7H_{15}$-Di*PP*ro-d4TDP 7a.** General procedure E; phosphoramidite **5a** (144 mg, 0.26 mmol), d4T monophosphate (110 mg, 0.18 mmol), and 4,5-dicyanoimidazole activator solution (1.04 μL, 0.26 mmol) in 4 mL of acetonitrile. Oxidation by addition of *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane, 47 μL, 0.26


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mmol). The product (108 mg, 0.14 mmol, 79%) was obtained as a colorless wad after ion exchange. $^1$H NMR (600 MHz, MeOH-$d_4$): $\delta$ [ppm] = 7.76 (s, 1H, H-6); 7.41−7.37 (m, 4H, H-c, H-c′); 7.08−7.04 (m, 4H, H-d, H-d′); 6.95−6.95 (m, 1H, H-1′); 6.39−6.38 (m, 1H, H-3′); 5.84−5.83 (m, 1H, H-2′); 5.12−5.09 (m, 4H, H-a, H-a′); 4.95 (bs, 1H, H-4′); 4.22−4.15 (m, 2H, H-5′); 2.58 (t, $^3J_{H,H}$ = 7.4 Hz, 2H, H-g′); 2.27 (s, 3H, $CH_3$); 1.89 (s, 3H, H-7); 1.73 (dt, $^3J_{H,H}$ = 7.4 Hz, $^3J_{H,H}$ = 7.4 Hz, 2H, H-h′); 1.45−1.29 (m, 8H, H-i′−H-l′); 0.92 (t, $^3J_{H,H}$ = 6.8 Hz, 3H, H-m′). $^{13}$C NMR (150 MHz, MeOH-$d_4$): $\delta$ [ppm] = 173.9 (C-f′); 171.2 (C-f); 166.7 (C-4); 152.8 (C-2); 152.5 (C-e, C-e′); 138.7 (C-6); 135.3 (C-3′); 135.0 (C-b′, C-b); 130.4 (2 ×d, $^4J_{C,P}$ = 4.8 Hz, $^4J_{C,P}$ = 4.8 Hz, C-c, C-c′); 127.6 (C-2′); 122.9 (C-d, C-d′); 112.1 (C-5); 90.8 (C-1′); 86.9 (d, $^3J_{C,P}$ = 9.0 Hz, C-4′); 70.2 (d, $^2J_{C,P}$ = 5.2 Hz, C-a, C-a′); 67.2 (d, $^3J_{C,P}$ = 5.5 Hz, C-5′); 35.0 (C-g′); 32.9, 30.1, 30.1, 23.7 (C-i′−C-l′); 26.0 (C-h′); 20.9 ($CH_3$); 14.4 (C-m′); 12.5 (C-7). $^{31}$P NMR (243 MHz, MeOH-$d_4$, decoupled): $\delta$/ppm = −12.07 (d, $^2J_{P,P}$ = 20.0 Hz, 1P, $P_\beta$); −12.92 (d, $^2J_{P,P}$ = 20.7 Hz, 1P, $P_\alpha$). HRMS (ESI$^+$, $m/z$) calcd for $C_{34}H_{42}N_2O_{14}P_2Na$: 787.2009 [M + Na]$^+$. Found: 787.1983. RP18-HPLC: method A, $t_R$ = 17.1 min.

**Ammonium-$CH_3$/$C_9H_{19}$-Di*PP*ro-d4TDP 7b.** General procedure E; phosphoramidite **5b** (165 mg, 0.29 mmol), d4T monophosphate (120 mg, 0.19 mmol), and 4,5-dicyanoimidazole activator solution (1.14 mL, 0.29 mmol) in 4 mL of acetonitrile. Oxidation by addition of *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane, 53 μL, 0.29 mmol). The product (106 mg, 0.031 mmol, 54%) was obtained as a colorless wad after ion exchange. $^1$H NMR (600 MHz, MeOH-$d_4$): $\delta$ [ppm] = 7.66 (s, 1H, H-6); 7.41−7.37 (m, 4H, H-c, H-c′); 7.08−7.04 (m, 4H, H-d, H-d′); 6.95−6.95 (m, 1H, H-1′); 6.39−6.38 (m, 1H, H-3′); 5.84−5.83 (m, 1H, H-2′); 5.12−5.09 (m, 4H, H-a, H-a′); 4.95 (bs, 1H, H-4′); 4.22−4.15 (m, 2H, H-5′); 2.58 (t, $^3J_{H,H}$ = 7.4 Hz, 2H, H-g′); 2.27 (s, 3H, $CH_3$); 1.89 (s, 3H, H-7); 1.72 (dt, $^3J_{H,H}$ = 7.4 Hz, $^3J_{H,H}$ = 7.5 Hz, 2H, H-h′); 1.44−1.30 (m, 12H, H-i′−H-n′); 0.91 (t, $^3J_{H,H}$ = 6.6 Hz, 3H, H-o′). $^{13}$C NMR (150 MHz, MeOH-$d_4$): $\delta$ [ppm] = 173.9 (C-f′); 171.1 (C-f); 166.7 (C-4); 152.8 (C-2); 152.5 (C-e, C-e′); 138.7 (C-6); 135.3 (C-3′); 135.0 (C-b′, C-b); 130.4 (2 ×d, $^4J_{C,P}$ = 4.5 Hz, $^4J_{C,P}$ = 5.0 Hz, C-c, C-c′); 127.6 (C-2′); 122.9 (C-d, C-d′); 112.1 (C-5); 90.8 (C-1′); 86.9 (d, $^3J_{C,P}$ = 9.4 Hz, C-4′); 70.2 (C-a, C-a′); 68.0 (d, $^3J_{C,P}$ = 6.2 Hz, C-5′); 35.0 (C-g′); 33.1, 30.6, 30.4, 30.2, 23.7 (C-i′−C-n′); 26.0 (C-h′); 20.9 ($CH_3$); 14.4 (C-o′); 12.5 (C-7). $^{31}$P NMR (243 MHz, MeOH-$d_4$, decoupled): $\delta$/ppm = −12.07 (d, $^2J_{P,P}$ = 20.6 Hz, 1P, $P_\beta$); −12.92 (d, $^2J_{P,P}$ = 20.5 Hz, 1P, $P_\alpha$). HRMS (ESI$^+$, $m/z$) calcd for $C_{36}H_{46}N_2O_{14}P_2Na$: 815.2322 [M + Na]$^+$. Found: 815.2312. RP18-HPLC: method A, $t_R$ = 18.5 min.

**Ammonium-$CH_3$/$C_{11}H_{23}$-Di*PP*ro-d4TDP 7c.** General procedure E; phosphoramidite **5c** (108 mg, 0.18 mmol), d4T monophosphate (77 mg, 0.12 mmol), and 4,5-dicyanoimidazole activator solution (720 μL, 0.18 mmol) in 4 mL of acetonitrile. Oxidation by addition of *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane, 33 μL, 0.18 mmol). The product (24 mg, 0.028 mmol, 23%) was obtained as a colorless wad after ion exchange. $^1$H NMR (600 MHz, MeOH-$d_4$): $\delta$ [ppm] = 7.66 (d, $^4J_{H,H}$ = 1.0 Hz, 1H, H-6); 7.41−7.37 (m, 4H, H-c, H-c′); 7.08−7.04 (m, 4H, H-d, H-d′); 6.94 (ddd, $^3J_{H,H}$ = 3.5 Hz, $^{3/4}J_{H,H}$ = 1.7 Hz, $^{3/4}J_{H,H}$ = 1.6 Hz, 1H, H-1′); 6.38 (ddd, $^3J_{H,H}$ = 6.0 Hz, $^3J_{H,H}$ = 3.2 Hz, $^4J_{H,H}$ = 3.2 Hz, 1H, H-3′); 5.83 (ddd, $^3J_{H,H}$ = 5.9 Hz, $^3J_{H,H}$ = 3.4 Hz, $^4J_{H,H}$ = 3.4 Hz, 1H, H-2′); 5.12−5.09 (m, 4H, H-a, H-a′); 4.94 (bs, 1H, H-4′); 4.23−4.15 (m, 2H, H-5′); 2.58 (t, $^3J_{H,H}$ = 7.4 Hz, 2H, H-g′); 2.27 (s, 3H, $CH_3$); 1.89 (d, $^4J_{H,H}$ = 1.0 Hz 3H, H-7); 1.73 (m, 2H, H-h′); 1.45−1.29 (m, 12H, H-i′−H-p′); 0.90 (t, $^3J_{H,H}$ = 7.1 Hz, 3H, H-q′). $^{13}$C NMR (150 MHz, MeOH-$d_4$): $\delta$ [ppm] = 173.8 (C-f′); 171.1 (C-f); 166.5 (C-4); 152.8 (C-2); 152.3 (C-e, C-e′); 138.7 (C-6); 135.3 (C-3′); 134.9 (C-b′, C-b); 130.4 (2 ×d, $^4J_{C,P}$ = 4.6 Hz, $^4J_{C,P}$ = 5.2 Hz, C-c, C-c′); 127.6 (C-2′); 122.9 (C-d, C-d′); 112.1 (C-5); 90.8 (C-1′); 86.9 (d, $^3J_{C,P}$ = 9.1 Hz, C-4′); 70.2 (C-a, C-a′); 68.1 (d, $^3J_{C,P}$ = 6.6 Hz, C-5′); 35.0 (C-g′); 33.1, 30.7, 30.6, 30.5, 30.4, 30.2, 23.7 (C-i′−C-p′); 26.0 (C-h′); 20.9 ($CH_3$); 14.4 (C-q′); 12.5 (C-7). $^{31}$P NMR (243 MHz, $D_2O$, decoupled): $\delta$/ppm = −12.08 (d, $^2J_{P,P}$ = 20.8 Hz, $P_\beta$); −12.92 (d, $^2J_{P,P}$ = 20.2 Hz, $P_\alpha$). HRMS (ESI$^-$, $m/z$) calcd for $C_{38}H_{49}N_2O_{14}P_2$: 819.2665 [M − H$^+$]$^-$. Found: 819.2604. RP18-HPLC: method A, $t_R$ = 19.5 min.

**Ammonium-$CH_3$/$CF_3$-Ph-Di*PP*ro-d4TDP 7d.** General procedure E; phosphoramidite **5d** (140 mg, 0.24 mmol), d4T monophosphate (100 mg, 0.16 mmol), and 4,5-dicyanoimidazole activator solution (960 μL, 0.24 mmol) in 4 mL of acetonitrile. Oxidation by addition of *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane, 44 μL, 0.24 mmol). The product (40 mg, 0.048 mmol, 30%) was obtained as a colorless wad after ion exchange. $^1$H NMR (600 MHz, MeOH-$d_4$): $\delta$ [ppm] = 8.38−8.37 (m, 2H, H-h′/H-i′); 7.90−7.89 (m, 2H, H-h′/H-i′); 7.66 (d, $^4J_{H,H}$ = 1.2 Hz, 1H, H-6); 7.48−7.39 (m, 4H, H-c′, H-c); 7.25−7.22 (m, 2H, H-d′); 7.09−7.07 (m, 2H, H-d); 6.96 (ddd, $^3J_{H,H}$ = 3.5 Hz, $^3J_{H,H}$ = 1.8 Hz, $^4J_{H,H}$ = 1.8 Hz, 1H, H-1′); 6.40−6.39 (m 1H, H-3′); 5.84 (ddd, $^3J_{H,H}$ = 6.0 Hz, $^3J_{H,H}$ = 3.4 Hz, $^4J_{H,H}$ = 3.4 Hz, 1H, H-2′); 5.16−5.11 (m, 4H, H-a, H-a′); 4.96 (bs, 1H, H-4′); 4.24−4.17 (m, 1H, H-5′); 2.25 (s, 3H, $CH_3$); 1.90 (s, 3H, H-7). $^{13}$C NMR (150 MHz, MeOH-$d_4$): $\delta$ [ppm] = 171.1 (C-f); 166.6 (C-4); 165.3 (C-f′); 152.9 (C-2); 152.4 (C-e/Ce′); 152.3 (C-e/C-e′); 138.7 (C-6); 135.9 (C-j′); 135.4 (C-b′); 135.3 (C-3′); 135.0 (C-b); 134.4 (C-g′); 131.8 (C-h′/C-i′); 130.5 (d, $^4J_{C,P}$ = 4.6 Hz, C-c′); 130.4 (d, $^4J_{C,P}$ = 5.5 Hz, C-c); 127.6 (C-2′); 126.9 (C-h′/C-i′); 124.3 ($CF_3$); 122.9 (C-d′); 122.9 (C-d); 112.0 (C-5); 90.8 (C-1′); 86.9 (d, $^3J_{C,P}$ = 9.5 Hz, C-4′); 70.2 (C-a, C-a′); 68.1 (C-5′); 20.6 ($CH_3$); 12.5 (C-7). $^{19}$F NMR (565 MHz, MeOH-$d_4$): $\delta$/ppm = −60.75. $^{31}$P NMR (243 MHz, $D_2O$, decoupled): $\delta$/ppm = −12.1 (d, $^2J_{P,P}$ = 20.4 Hz, $P_\beta$); −12.9 (d, $^2J_{P,P}$ = 19.8 Hz, $P_\alpha$). HRMS (ESI$^-$, $m/z$) calcd for $C_{34}H_{30}F_3N_2O_{14}P_2$: 809.1130 [M − H$^+$]$^-$. Found: 809.1096. Rp18-HPLC: method A, $t_R$ = 15.8 min.

**Tetrabutylmmonium-$CH_3$/$CH_3$-Ph-Di*PP*ro-d4TDP 7e.** General procedure E; phosphoramidite **5e** (190 mg, 0.35 mmol), d4T monophosphate (150 mg, 0.24 mmol), and 4,5-dicyanoimidazole activator solution (1.20 mL, 0.30 mmol) in 6 mL of acetonitrile. Oxidation by addition of *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane, 64 μL, 0.35 mmol). The product (115 mg, 0.115 mmol, 48%) was obtained as a colorless wad. $^1$H NMR (600 MHz, $D_2O$): $\delta$ [ppm] = 8.07−8.06 (m 2H, H-h′); 7.67 (d, $^4J_{H,H}$ = 1.1 Hz, 1H, H-6); 7.47−7.39 (m 4H, H-c, H-c′); 7.38−7.37 (m, 2H, H-d/H-d′); 7.20−7.18 (m, 2H, H-d/H-d′); 7.09−7.07 (m, 2H, H-i′); 6.96 (ddd, $^3J_{H,H}$ = 3.6 Hz, $^3J_{H,H}$ = 1.7 Hz, $^4J_{H,H}$ = 1.7 Hz, 1H, H-1′); 6.40 (ddd, $^3J_{H,H}$ = 6.0 Hz, $^3J_{H,H}$ = 3.2 Hz, $^4J_{H,H}$ = 3.2 Hz, 1H, H-3′); 5.84 (ddd, $^3J_{H,H}$ = 6.0 Hz, $^3J_{H,H}$ = 3.4 Hz, $^4J_{H,H}$ = 3.4 Hz, 1H, H-2′); 5.16−5.11 (m, 4H, H-a, H-a′); 4.96 (bs, 1H, H-4′); 4.25−4.17 (m, 2H, H-5′); 3.24−3.21 (m, 8H, H-A); 2.45 (s, 3H, H-k′); 2.25 (s, 3H, $CH_3$); 1.90 (d, $^4J_{H,H}$ = 1.1 Hz, 3H, H-7); 1.68−1.62 (m, 8H, H-B); 1.41 (dq, $^3J_{H,H}$ = 7.4 Hz, $^3J_{H,H}$ = 7.4 Hz, 8H, H-C); 1.02 (t, $^3J_{H,H}$ = 7.4 Hz, 12H, H-D). $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ [ppm] = 171.0 (C-f); 166.6 (C-4); 166.5 (C-f′); 152.8 (C-2); 152.6 (C-e/C-e′); 152.4 (C-e/C-e′); 146.2 (C-g′); 138.7 (C-6); 135.3 (C-3′); 135.1 (dd, $^3J_{C,P}$ = 7.5 Hz, $^5J_{C,P}$ = 3.3 Hz, C-b/C-b′); 135.1 (dd, $^3J_{C,P}$ = 7.2 Hz, $^5J_{C,P}$ = 2.8 Hz, C-b/C-b′); 131.2 (C-h′); 130.5 (C-i′); 130.5 (d, $^4J_{C,P}$ = 5.5 Hz, C-c′); 130.4 (d, $^4J_{C,P}$ = 4.7 Hz, C-c); 128.0 (C-j′); 127.6 (C-2′); 123.0 (C-d); 122.9 (C-d′); 112.1 (C-5); 90.8 (C-1′); 87.0 (d, $^3J_{C,P}$ = 9.4 Hz, C-4′); 70.2 (d, $^2J_{C,P}$ = 5.6 Hz, C-a); 70.2 (d, $^2J_{C,P}$ = 5.0 Hz, C-a′); 68.1 (d, $^2J_{C,P}$ = 6.2 Hz, C-5′); 59.5, 59.5, 59.5 (C-A); 24.8 (C-B); 21.7 (C-k′); 20.9 ($CH_3$); 20.7 (C-C); 13.9 (C-D); 12.5 (C-7). $^{31}$P NMR (243 MHz, $D_2O$, decoupled): $\delta$/ppm = −12.1 (d, $^2J_{P,P}$ = 20.6 Hz, $P_\beta$); −12.9 (d, $^2J_{P,P}$ = 20.8 Hz, $P_\alpha$). HRMS (ESI$^-$, $m/z$) calcd for $C_{34}H_{33}N_2O_{14}P_2$: 755.1413 [M − H$^+$]$^-$. Found: 755.1413. RP18-HPLC: method A, $t_R$ = 15.4 min.

**Ammonium-$C_4H_9$/$C_7H_{15}$-Di*PP*ro-d4TDP 8a.** General procedure E; phosphoramidite **5f** (112 mg, 0.19 mmol), d4T monophosphate (79 mg, 0.13 mmol), and 4,5-dicyanoimidazole activator solution (760 μL, 0.19 mmol) in 3 mL of acetonitrile. Oxidation by addition of *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane, 35 μL, 0.19 mmol). The product (76 mg, 0.092 mmol, 71%) was obtained as a colorless wad after ion exchange. $^1$H NMR (600 MHz, MeOH-$d_4$): $\delta$ [ppm] = 7.76 (s, 1H, H-6); 7.40−7.37 (m, 4H, H-c, H-c′); 7.06−7.04 (m, 4H, H-d, H-d′); 6.95 (bs, 1H, H-1′); 6.39−6.38 (m, 1H, H-3′); 5.84−5.83 (m, 1H, H-2′); 5.11−5.09 (m, 4H, H-a, H-a′); 4.95 (bs, 1H, H-4′); 4.22−4.15 (m, 2H, H-5′); 2.60−2.56 (m, 4H, H-g, H-g′); 1.89 (s, 3H, H-7); 1.71 (dt, $^3J_{H,H}$ = 7.7 Hz, $^3J_{H,H}$ = 7.5 Hz, 4H, H-h, H-h′); 1.48−1.30 (m, 10H, H-i, H-i′−H-l′); 0.99 (t, $^3J_{H,H}$ = 7.4 Hz, 3H, H-j); 0.92 (t, $^3J_{H,H}$ = 6.7 Hz, 3H, H-m′). $^{13}$C NMR (150 MHz, MeOH-$d_4$): $\delta$


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J. Med. Chem. 2015, 58, 6114−6130

[ppm] = 173.8 (C-f, C-f′); 166.7 (C-4); 152.4 (C-2); 152.4 (C-e, C-e′); 138.7 (C-6); 135.3 (C-3′); 134.9 (d, $^{3}J_{C,P}$ = 2.4 Hz, C-b′/C-b); 134.9 (d, $^{3}J_{C,P}$ = 3.2 Hz, C-b′/C-b); 130.4 (d, $^{4}J_{C,P}$ = 4.5 Hz, C-c/C-c′); 127.6 (C-2′); 122.9 (C-d, C-d′); 112.1 (C-5); 90.8 (C-1′); 86.9 (d, $^{3}J_{C,P}$ = 9.0 Hz, C-4′); 70.2 (2 ×d, $^{2}J_{C,P}$ = 5.0 Hz, $^{2}J_{C,P}$ = 4.2 Hz, C-a, C-a′); 68.1 (d, $^{3}J_{C,P}$ = 6.1 Hz, C-5′); 35.0 (C-g′); 34.8 (C-g); 32.9, 30.1, 30.1, 23.7, (C-i′−C-l′); 28.1 (C-h); 26.0 (C-h′); 23.5 (C-i); 14.4 (C-m′); 14.1 (C-j); 12.5 (C-7). $^{31}$P NMR (243 MHz, MeOH-$d_4$, decoupled): $\delta$/ppm = −12.07 (d, $^{2}J_{P,P}$ = 20.5 Hz, 1P, $P_\beta$); −12.91 (d, $^{2}J_{P,P}$ = 20.5 Hz, 1P, $P_\alpha$). HRMS (ESI$^+$, $m/z$) calcd for $C_{37}H_{48}N_2O_{14}P_2Na$: 829.2478 [M + Na]$^+$. Found: 829.2411. RP18-HPLC: method A, $t_R$ = 18.8 min.

**Ammonium-$C_4H_9$/$C_9H_{19}$-Di*PP*ro-d4TDP 8b.** General procedure E; phosphoramidite **5g** (164 mg, 0.27 mmol), d4T monophosphate (111 mg, 0.18 mmol), and 4,5-dicyanoimidazole activator solution (1.07 mL, 0.27 mmol) in 4 mL of acetonitrile. Oxidation by addition of *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane, 49 μL, 0.27 mmol). The product (115 mg, 0.14 mmol, 75%) was obtained as a colorless wad after ion exchange. $^1$H NMR (600 MHz, MeOH-$d_4$): $\delta$ [ppm] = 7.65 (s, 1H, H-6); 7.40−7.37 (m, 4H, H-c, H-c′); 7.06−7.04 (m, 4H, H-d, H-d′); 6.95−9.95 (m, 1H, H-1′); 6.38−6.37 (m, 1H, H-3′); 5.84−5.83 (m, 1H, H-2′); 5.11−5.09 (m, 4H, H-a, H-a′); 4.95 (bs, 1H, H-4′); 4.22−4.14 (m, 2H, H-5′); 2.58, 2.58 (2 × t, $^{3}J_{H,H}$ = 7.4 Hz, $^{3}J_{H,H}$ = 7.3 Hz 4H, H-g, H-g′); 1.89 (s, 3H, H-7); 1.72 (dt, $^{3}J_{H,H}$ = 7.6 Hz, $^{3}J_{H,H}$ = 7.4 Hz, 4H, H-h, H-h′); 1.49−1.31 (m, 14H, H-i, H-i′−H-n′); 0.99 (t, $^{3}J_{H,H}$ = 7.4 Hz, 3H, H-j); 0.90 (t, $^{3}J_{H,H}$ = 6.7 Hz, 3H, H-o′). $^{13}$C NMR (150 MHz, MeOH-$d_4$): $\delta$ [ppm] = 173.8 (C-f/C-f′); 173.8 (C-f/C-f′); 166.5 (C-4); 152.8 (C-2); 152.4 (C-e/C-e′); 152.4 (C-e/C-e′); 138.7 (C-6); 135.3 (C-3′); 134.9 (d, $^{3}J_{C,P}$ = 7.7 Hz, C-b′/C-b); 134.9 (d, $^{3}J_{C,P}$ = 6.8 Hz, C-b′/C-b); 130.4 (d, $^{4}J_{C,P}$ = 3.8 Hz, C-c/C-c′); 127.6 (C-2′); 122.9 (C-d, C-d′); 112.1 (C-5); 90.8 (C-1″); 86.9 (d, $^{3}J_{C,P}$ = 9.3 Hz, C-4′); 70.3 (d, $^{2}J_{C,P}$ = 5.2 Hz, C-a/C-a′); 70.2 (d, $^{2}J_{C,P}$ = 5.3 Hz, C-a/C-a′); 68.1 (d, $^{3}J_{C,P}$ = 6.3 Hz, C-5′); 35.0 (C-g′); 34.8 (C-g); 33.0, 30.6, 30.4, 30.2, 23.7, (C-i′−C-n′); 28.1 (C-h); 26.0 (C-h′); 23.2 (C-i); 14.4 (C-o′); 14.1 (C-j); 12.5 (C-7). $^{31}$P NMR (243 MHz, MeOH-$d_4$, decoupled): $\delta$/ppm = −12.06 (d, $^{2}J_{P,P}$ = 20.5 Hz, 1P, $P_\beta$); −12.88 (d, $^{2}J_{P,P}$ = 20.5 Hz, 1P, $P_\alpha$). HRMS (ESI$^-$, $m/z$) calcd for $C_{39}H_{51}N_2O_{14}P_2$: 833.2821 [M − H$^+$]$^-$. Found: 833.2638. RP18-HPLC: method A, $t_R$ = 20.2 min.

**Ammonium-$C_4H_9$/$C_{11}H_{23}$-Di*PP*ro-d4TDP 8c.** General procedure E; phosphoramidite **5h** (42 mg, 0.058 mmol), d4T monophosphate (33 mg, 0.053 mmol), and 4,5-dicyanoimidazole activator solution (230 μL, 0.053 mmol) in 2 mL of acetonitrile. Oxidation by addition of *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane, 11 μL, 0.058 mmol). The product (33 mg, 0.038 mmol, 71%) was obtained as a colorless wad after ion exchange. $^1$H NMR (600 MHz, $D_2O$): $\delta$ [ppm] = 7.66 (d, $^{4}J_{H,H}$ = 1.2 Hz, 1H, H-6); 7.40−7.37 (m, 4H, H-c, H-c′); 7.06−7.04 (m, 4H, H-d, H-d′); 6.95 (ddd, $^{3}J_{H,H}$ = 3.5 Hz, $^{3}J_{H,H}$ = 1.7 Hz, $^{4}J_{H,H}$ = 1.7 Hz, 1H, H-1′); 6.38 (ddd, $^{3}J_{H,H}$ = 6.0 Hz, $^{3}J_{H,H}$ = 3.4 Hz, $^{4}J_{H,H}$ = 3.4 Hz, 1H, H-3′); 5.83 (ddd, $^{3}J_{H,H}$ = 5.6 Hz, $^{3}J_{H,H}$ = 1.8 Hz, $^{4}J_{H,H}$ = 1.8 Hz, 1H, H-2′); 5.11−5.08 (m, 4H, H-a, H-a′); 4.95 (bs, 1H, H-4′); 4.23−4.15 (m, 2H, H-5′); 2.60−2.56 (m, 4H, H-g, H-g′); 1.89 (d, $^{4}J_{H,H}$ = 1.1 Hz, 3H, H-7); 1.76−1.69 (m, 4H, H-h, H-h′); 1.49−1.1.27 (m, 18H, H-i, H-i′-H-p′); 0.99 (t, $^{3}J_{H,H}$ = 7.4 Hz, 3H, H-j); 0.99 (t, $^{3}J_{H,H}$ = 6.7 Hz, 3H, H-q′). $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ [ppm] = 173.6 (C-f, C-f′); 166.5 (C-4); 152.8 (C-2); 152.3 (C-e, C-e′); 138.7 (C-6); 133.9 (C-3′); 134.8 (C-b′, C-b); 130.4 (C-c, C-c′); 127.3 (C-2′); 122.9 (C-d, C-d′); 112.1 (C-5); 90.8 (C-1′); 86.9 (C-4′); 70.2 (C-a, C-a′); 68.1 (C-5′); 35.0 (C-g′); 34.8 (C-g); 32.9, 30.7, 30.6, 30.4, 30.2, 23.5, (C-i′−C-p′); 28.0 (C-h); 25.8 (C-h′); 22.9 (C-i); 14.2 (C-q′); 13.8 (C-j); 12.2 (C-7). $^{31}$P NMR (243 MHz, $D_2O$, decoupled): $\delta$/ppm = −12.1 (d, $^{2}J_{P,P}$ = 20.6 Hz, $P_\beta$); −12.9 (d, $^{2}J_{P,P}$ = 21.5 Hz, $P_\alpha$). HRMS (ESI$^+$, $m/z$) calcd for $C_{41}H_{53}N_2O_{14}P_2Na$: 885.3104 [M + Na]$^+$. Found: 885.3022. RP18-HPLC: method A, $t_R$ = 21.4 min.

**Ammonium-$C_4H_9$/$CF_3$-Ph-Di*PP*ro-d4TDP 8d.** General procedure E; phosphoramidite **5i** (180 mg, 0.29 mmol), d4T monophosphate (121 mg 0.19 mmol), and 4,5-dicyanoimidazole activator solution (950 μL, 0.24 mmol) in 5 mL of acetonitrile. Oxidation by addition of *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane, 53 μL, 0.29 mmol). The product (89 mg, 0.10 mmol, 53%) was obtained as a colorless wad after ion exchange. $^1$H NMR (600 MHz, MeOH-$d_4$): $\delta$ [ppm] = 8.38−8.37 (m, 2H, H-h′/H-i′); 7.90−7.89 (m, 2H, H-h′/H-i′); 7.67 (d, $^{4}J$ = 1.0 Hz, 1H, H-6); 7.47−7.44 (m, 2H, H-c′); 7.42−7.38 (m, 2H, H-c); 7.24−7.22 (m, 2H, H,d′); 7.07−7.05 (m, 2H, H-d); 6.96 (ddd, $^{3}J_{H,H}$ = 3.5 Hz, $^{3}J_{H,H}$ = 1.7 Hz, $^{4}J_{H,H}$ = 1.7 Hz, 1H, H-1′); 6.41−6.39 (m 1H, H-3′); 5.85−5.84 (m, 1H, H-2′); 5.16−5.11 (m, 4H, H-a, H-a′); 4.96 (bs, 1H, H-4′); 4.25−4.17 (m, 1H, H-5′); 2.56 (t, $^{3}J_{H,H}$ = 7.4 Hz, 2H, H-g); 1.90 (s, 3H, H-7); 1.70−1.66 (m, 2H, H-h); 1.46−1.39 (m, 2H, H-i); 0.96 (t, $^{3}J_{H,H}$ = 7.4 Hz, 3H, H-j). $^{13}$C NMR (150 MHz, MeOH-$d_4$): $\delta$ [ppm] = 173.9 (C-f); 166.9 (C-4); 165.3 (C-f′); 152.9 (C-2); 152.6 (C-e); 152.4 (C-e′); 138.9 (C-6); 136.1 (C-j′); 135.6 (C-b′); 135.1 (C-b); 135.0 (C-3′); 134.5 (C-g′); 131.8 (C-h′/C-i′); 130.5 (d, $^{4}J_{C,P}$ = 5.5 Hz, C-c′); 130.4 (d, $^{4}J_{C,P}$ = 5.6 Hz, C-c); 127.6 (C-2′); 126.9 (C-h′/C-i′); 124.3 ($CF_3$); 122.9 (C-d′); 122.9 (C-d); 112.4 (C-5); 90.6 (C-1′); 87.0 (d, $^{3}J_{C,P}$ = 9.3 Hz, C-4′); 70.2 (C-a, C-a′); 68.1 (C-5′); 34.7 (C-g); 28.0 (C-h); 23.1 (C-i); 14.0 (C-j); 12.5 (C-7). $^{19}$F NMR (565 MHz, MeOH-$d_4$): $\delta$/ppm = −60.75. $^{31}$P NMR (243 MHz, MeOH-$d_4$, decoupled): $\delta$/ppm = −12.1 (bs, $P_\beta$); −12.9 (d, $^{2}J_{P,P}$ = 20.8 Hz, $P_\alpha$). $^{19}$F NMR (565 MHz, MeOH-$d_4$): $\delta$/ppm = −60.75. HRMS (ESI$^-$, $m/z$) calcd for $C_{37}H_{36}F_3N_2O_{14}P_2$: 851.1559 [M − H$^+$]$^-$. Found: 851.1597. RP18-HPLC: method A, $t_R$ = 17.4 min.

**Ammonium-$C_4H_9$/$CH_3$-Ph-Di*PP*ro-d4TDP 8e.** General procedure E; phosphoramidite **5j** (123 mg, 0.21 mmol), d4T monophosphate (89 mg, 0.14 mmol), and 4,5-dicyanoimidazole activator solution (860 μL, 0.21 mmol) in 4 mL of acetonitrile. Oxidation by addition of *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane, 39 μL, 0.21 mmol). The product (36 mg, 0.044 mmol, 31%) was obtained as a colorless wad after ion exchange. $^1$H NMR (600 MHz, $D_2O$): $\delta$ [ppm] = 8.07−8.06 (m 2H, H-h′); 7.67 (d, $^{4}J_{H,H}$ = 1.2 Hz, 1H, H-6); 7.45−7.38 (m 6H, H-c, H-c′, H-d/H-d′); 7.20−7.18 (m, 2H, H-d/H-d′); 7.07−7.04 (m, 2H, H-i′); 6.96 (ddd, $^{3}J_{H,H}$ = 3.5 Hz, $^{3}J_{H,H}$ = 1.7 Hz, $^{4}J_{H,H}$ = 1.7 Hz, 1H, H-1′); 6.40 (ddd, $^{3}J_{H,H}$ = 6.0 Hz, $^{3}J_{H,H}$ = 3.3 Hz, $^{4}J_{H,H}$ = 3.3 Hz, 1H, H-3′); 5.84 (ddd, $^{3}J_{H,H}$ = 5.9 Hz, $^{3}J_{H,H}$ = 3.5 Hz, $^{4}J_{H,H}$ = 3.5 Hz, 1H, H-2′); 5.15−5.10 (m, 4H, H-a, H-a′); 4.96 (bs, 1H, H-4′); 4.25−4.16 (m, 2H, H-5′); 2.56 (t, 2H, H-g); 2.46 (s, 3H, H-k′); 1.90 (d, $^{4}J_{H,H}$ = 1.1 Hz, 3H, H-7); 1.72−1.65 (m, 2H, H-h); 1.41 (m, 2H, H-i); 0.96 (t, $^{3}J_{H,H}$ = 7.4 Hz, 3H, H-j). $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ [ppm] = 173.6 (C-f); 166.6 (C-4); 166.5 (C-f′); 152.7 (C-2); 152.5 (C-e/C-e′); 152.3 (C-e/C-e′); 146.2 (C-g′); 138.6 (C-6); 135.2 (C-3′); 135.1 (C-b/C-b′); 134.9 (C-b/C-b′); 131.2 (C-h′); 131.2 (C-c, C-c′); 130.5 (C-i′); 130.4 (C-j′); 127.6 (C-2′); 123.0 (C-d); 122.9 (C-d′); 112.1 (C-5); 90.8 (C-1′); 87.0 (d, $^{3}J_{C,P}$ = 9.4 Hz, C-4′); 70.3 (C-a, C-a′); 68.1 (C-5′); 34.7 (C-g); 27.9 (C-h); 23.2 (C-i); 21.6 (C-k′); 13.8 (C-j); 12.4 (C-7). $^{31}$P NMR (243 MHz, $D_2O$, decoupled): $\delta$/ppm = −12.1 (d, $^{2}J_{P,P}$ = 20.2 Hz, $P_\beta$); −12.9 (d, $^{2}J_{P,P}$ = 21.0 Hz, $P_\alpha$). HRMS (ESI$^-$, $m/z$) calcd for $C_{37}H_{39}N_2O_{14}P_2$: 797.1882 [M − H$^+$]$^-$. Found: 797.1855. RP18-HPLC: method A, $t_R$ = 17.0 min.

**Ammonium-$CH_3$/$C_7H_{15}$-Di*PP*ro-AZTDP 9a.** General procedure E; phosphoramidite **5a** (130 mg, 0.24 mmol), AZT monophosphate (82 mg, 0.16 mmol), and 4,5-dicyanoimidazole activator solution (960 μL, 0.24 mmol) in 3 mL of acetonitrile. Oxidation by addition of *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane, 44 μL, 0.24 mmol). The product (79 mg, 0.096 mmol, 60%) was obtained as a colorless wad after ion exchange. $^1$H NMR (600 MHz, MeOH-$d_4$): $\delta$ [ppm] = 7.721 (s, 1H, H-6); 7.42−7.40 (m, 4H, H-c, H-c′); 7.08−7.05 (m, 4H, H-d, H-d′); 6.21 (dd, $^{3}J_{H,H}$ = 6.8 Hz, $^{3}J_{H,H}$ = 6.8 Hz, 1H, H-1′); 5.14−5.11 (m, 4H, H-a, H-a′); 4.41−4.39 (m, 1H, H-3′); 4.21−4.18 (m, 1H, H-5′a); 4.13−4.10 (m, 1H, H-5′b); 4.04−4.03 (m, 1H, H-4′); 2.58 (t, $^{3}J_{H,H}$ = 7.4 Hz, 2H, H-g′); 2.36−2.31 (m, 2H, H-2′a); 2.30−2.26 (m, 2H, H-2′b); 2.27 (s, 3H, $CH_3$); 1.90 (s, 3H, H-7); 1.73 (dt, $^{3}J_{H,H}$ = 7.4 Hz, $^{3}J_{H,H}$ = 7.4 Hz, 2H, H-h′); 1.44−1.30 (m, 8H, H-i′−H-l′); 0.92 (t, $^{3}J_{H,H}$ = 6.8 Hz, 3H, H-m′). $^{13}$C NMR (150 MHz, MeOH-$d_4$): $\delta$ [ppm] = 173.8 (C-f′); 171.0 (C-f); 166.4 (C-4); 152.5 (C-e/Ce′); 152.4 (C-e/Ce′); 152.3 (C-2); 137.7 (C-6); 134.9 (C-b′, C-b); 130.5 (d, $^{4}J_{C,P}$ = 3.2 Hz, C-c/C-c′); 130.4 (d, $^{4}J_{C,P}$ = 3.3 Hz, C-c/C-c′); 122.9 (C-d, C-d′); 112.2 (C-5); 85.8 (C-1′); 84.4 (d, $^{3}J_{C,P}$ = 9.4 Hz, C-4′); 70.3 (d, $^{2}J_{C,P}$ = 5.2 Hz, C-a/C-a′); 67.2 (d, $^{3}J_{C,P}$ = 5.5 Hz, C-5′); 62.5 (C-3′); 38.0 (C-2′); 35.0 (C-g′); 32.9, 30.1, 30.1, 23.7 (C-i′−C-l′); 26.0 (C-



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h′); 20.9 ($CH_3$); 14.4 (C-m′); 12.6 (C-7). $^{31}$P NMR (243 MHz, MeOH-$d_4$, decoupled): $\delta$/ppm = −12.14 (d, $^2J_{P,P}$ = 20.1 Hz, 1P, $P_\beta$); −12.76 (d, $^2J_{P,P}$ = 20.3 Hz, 1P, $P_\alpha$). HRMS (ESI$^+$, $m/z$) calcd for $C_{34}H_{44}N_5O_{14}P_2$: 808.2355 [M + H$^+$]$^+$. Found: 808.2354. RP18-HPLC: method B, $t_R$ = 17.4 min.

**Ammonium-$CH_3$/$C_9H_{19}$-Di*PP*ro-AZTDP 9b.** General procedure E; phosphoramidite **5b** (100 mg, 0.18 mmol), AZT monophosphate (73 mg, 0.12 mmol), and 4,5-dicyanoimidazole activator solution (700 μL, 0.18 mmol) in 3 mL of acetonitrile. Oxidation by addition of *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane, 32 μL, 0.18 mmol). The product (45 mg, 0.053 mmol, 45%) was obtained as a colorless wad after ion exchange. $^1$H NMR (600 MHz, MeOH-$d_4$): $\delta$ [ppm] = 7.72 (s, 1H, H-6); 7.42−7.40 (m, 4H, H-c, H-c′); 7.08−7.05 (m, 4H, H-d, H-d′); 6.21 (dd, $^3J_{H,H}$ = 6.8 Hz, $^3J_{H,H}$ = 6.8 Hz, 1H, H-1′); 5.14−5.11 (m, 4H, H-a, H-a′); 4.40 (dt, $^3J_{H,H}$ = 6.6 Hz, $^3J_{H,H}$ = 6.8 Hz, 1H, H-3′); 4.21−4.18 (m, 1H, H-5′a); 4.13−4.10 (m, 1H, H-5′b); 4.04−4.03 (m, 1H, H-4′); 2.57 (t, $^3J_{H,H}$ = 7.4 Hz, 2H, H-g′); 2.36−2.31 (m, 2H, H-2′a); 2.30−2.26 (m, 2H, H-2′b); 2.27 (s, 3H, $CH_3$); 1.90 (s, 3H, H-7); 1.73 (dt, $^3J_{H,H}$ = 7.4 Hz, $^3J_{H,H}$ = 7.4 Hz, 2H, H-h′); 1.45−1.29 (m, 12H, H-i′−H-n′); 0.99 (t, $^3J_{H,H}$ = 7.4 Hz, 3H, H-j); 0.90 (t, $^3J_{H,H}$ = 6.8 Hz, 3H, H-o′). $^{13}$C NMR (150 MHz, MeOH-$d_4$): $\delta$ [ppm] = 173.7 (C-f′); 170.1 (C-f); 166.3 (C-4); 152.5 (C-e, Ce′); 152.4 (C-2); 137.8 (C-6); 134.9 (C-b′, C-b); 130.5 (C-c, C-c′); 122.9 (C-d, C-d′); 112.1 (C-5); 85.8 (C-1′); 84.4 (d, $^3J_{C,P}$ = 9.6 Hz, C-4′); 70.3 (d, $^2J_{C,P}$ = 6.4 Hz, C-a/C-a′); 67.3 (d, $^3J_{C,P}$ = 5.8 Hz, C-5′); 62.5 (C-3′); 38.0 (C-2′); 35.0 (C-g′); 33.0, 30.6, 30.4, 30.2, 23.8 (C-i′−C-n′); 26.0 (C-h′); 14.5 (C-o′); 12.6 (C-7). $^{31}$P NMR (243 MHz, MeOH-$d_4$, decoupled): $\delta$/ppm = −12.16 (d, $^2J_{P,P}$ = 20.2 Hz, 1P, $P_\beta$); −12.79 (d, $^2J_{P,P}$ = 19.8 Hz, 1P, $P_\alpha$). HRMS (ESI$^+$, $m/z$) calcd for $C_{36}H_{48}N_5O_{14}P_2$: 863.2668 [M + H$^+$]$^+$. Found: 836.2854. RP18-HPLC: method B, $t_R$ = 18.6 min.

**Ammonium-$CH_3$/$C_{11}H_{23}$-Di*PP*ro-AZTDP 9c.** General procedure E; phosphoramidite **5c** (125 mg, 0.21 mmol), AZT monophosphate (86 mg, 0.14 mmol), and 4,5-dicyanoimidazole activator solution (830 μL, 0.21 mmol) in 4 mL of acetonitrile. Oxidation by addition of *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane, 38 μL, 0.21 mmol). The product (58 mg, 0.066 mmol, 48%) was obtained as a colorless wad after ion exchange. $^1$H NMR (600 MHz, MeOH-$d_4$): $\delta$ [ppm] = 7.72 (s, 1H, H-6); 7.42−7.40 (m, 4H, H-c, H-c′); 7.08−7.05 (m, 4H, H-d, H-d′); 6.21 (dd, $^3J_{H,H}$ = 6.8 Hz, $^3J_{H,H}$ = 6.8 Hz, 1H, H-1′); 5.14−5.12 (m, 4H, H-a, H-a′); 4.4 (dt, $^3J_{H,H}$ = 6.5 Hz, $^3J_{H,H}$ = 6.7 Hz, 1H, H-3′); 4.21−4.18 (m, 1H, H-5′a); 4.13−4.10 (m, 1H, H-5′b); 4.04−4.03 (m, 1H, H-4′); 2.58 (d, $^3J_{H,H}$ = 7.4 Hz, 1H, H-g′); 2.36−2.26 (m, 2H, H-2′a, H-2′b); 2.27 (s, 3H, $CH_3$); 1.90 (s, 3H, H-7); 1.76−1.71 (m, 2H, H-h′); 1.45−1.25 (m, 16H, H-i′−H-p′); 0.90 (t, $^3J_{H,H}$ = 6.9 Hz, 3H, H-q′). $^{13}$C NMR (150 MHz, MeOH-$d_4$): $\delta$ [ppm] = 173.8 (C-f′); 171.0 (C-f); 166.3 (C-4); 152.5 (C-e, Ce′); 152.3 (C-2); 137.8 (C-6); 134.8 (C-b′, C-b); 130.5 (2 ×d, $^4J_{C,P}$ = 2.3 Hz, C-c, C-c′); 122.9 (C-d, C-d′); 112.2 (C-5); 85.8 (C-1′); 84.4 (d, $^3J_{C,P}$ = 8.8 Hz, C-4′); 70.3 (d, $^2J_{C,P}$ = 5.3 Hz, C-a/C-a′); 67.3 (d, $^3J_{C,P}$ = 5.8 Hz, C-5′); 62.5 (C-3′); 38.0 (C-2′); 35.0 (C-g′); 33.1, 30.7, 30.6, 30.5, 30.4, 30.2, 23.7 (C-i′−C-p′); 26.0 (C-h′); 20.9 ($CH_3$); 12.6 (C-7). $^{31}$P NMR (243 MHz, MeOH-$d_4$, decoupled): $\delta$/ppm = −12.17 (d, $^2J_{P,P}$ = 20.5 Hz, 1P, $P_\beta$); −12.8 (d, $^2J_{P,P}$ = 20.4 Hz, 1P, $P_\alpha$). HRMS (ESI$^+$, $m/z$) calcd for $C_{38}H_{51}N_5O_{14}P_2Na$: 864.2981 [M + Na]$^+$. Found: 864.2982. RP18-HPLC: method B, $t_R$ = 20.1 min.

**Ammonium-$CH_3$/Ph-$CF_3$-Di*PP*ro-AZTDP 9d.** General procedure E; phosphoramidite **5d** (140 mg, 0.24 mmol), AZT monophosphate (98 mg, 0.16 mmol), and 4,5-dicyanoimidazole activator solution (940 μL, 0.24 mmol) in 4 mL of acetonitrile. Oxidation by addition of *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane, 44 μL, 0.24 mmol). The product (83 mg, 0.095 mmol, 61%) was obtained as a colorless wad after ion exchange. $^1$H NMR (600 MHz, MeOH-$d_4$): $\delta$ [ppm] = 8.38−8.36 (m, 2H, H-h′/H-i′); 7.90−7.89 (m, 2H, H-h′/H-i′); 7.73 (s, 1H, H-6); 7.50−7.48 (m, 2H, H-c′); 7.45−7.42 (m, 2H, H-c); 7.25−7.23 (m, 2H, H-d′); 7.10−7.08 (m, 2H, H-d); 6.22 (dd, $^3J_{H,H}$ = 6.8 Hz, $^3J_{H,H}$ = 6.8 Hz, 1H, H-1′); 5.18−5.14 (m, 4H, H-a, H-a′); 4.43−4.40 (m, 1H, H-3′); 4.23−4.20 (m, 1H, H-5′a); 4.15−4.12 (m, 1H, H-5′b); 4.06−4.04 (m, 1H, H-4′); 2.36−2.26 (m, 2H, H-2′a, H-2′b); 2.25 (s, 3H, $CH_3$); 1.90 (s, 3H, H-7). $^{13}$C NMR (150 MHz, MeOH-$d_4$): $\delta$ [ppm] = 171.0 (C-f); 166.4 (C-4); 165.4(C-f′); 152.4 (C-2); 152.3 (C-e); 152.3 (C-e′); 137.8 (C-6); 135.9 (C-j′); 135.3 (C-b′); 135.1 (C-b); 134.4 (C-g′); 131.8 (C-h′/C-i′); 130.6 (d, $^5J_{C,P}$ = 4.2 Hz, C-c′); 130.1 (d, $^5J_{C,P}$ = 2.5 Hz, C-c); 126.5 (C-h′/C-i′); 124.4 ($CF_3$); 123.0 (C-d′); 122.9 (C-d); 112.2 (C-5); 85.8 (C-1′); 84.4 (d, $^3J_{C,P}$ = 9.5 Hz, C-4′); 70.4 (d, $^2J_{C,P}$ = 5.3 Hz, C-a/C-a′); 70.3 (d, $^2J_{C,P}$ = 5.5 Hz, C-a/C-a′); 67.3 (d, $^3J_{C,P}$ = 5.8 Hz, C-5′); 62.5 (C-3′); 38.0 (C-2′); 20.9 ($CH_3$); 12.6 (C-7). $^{17}$F NMR (565 MHz, MeOH-$d_4$): $\delta$/ppm = −64.7. $^{31}$P NMR (243 MHz, MeOH-$d_4$, decoupled): $\delta$/ppm = −12.14 (d, $^2J_{P,P}$ = 20.1 Hz, 1P, $P_\beta$); −12.68−12.83 (m, 1P, $P_\alpha$). HRMS (ESI$^+$, $m/z$) calcd for $C_{34}H_{33}F_3N_5O_{14}P_2$: 854.1451 [M + H$^+$]$^+$. Found: 854.1442. RP18-HPLC: method B, $t_R$ = 16.4 min.

**Ammonium-$C_4H_9$/$C_7H_{15}$-Di*PP*ro-AZTDP 10a.** General procedure E; phosphoramidite **5f** (132 mg, 0.23 mmol), AZT monophosphate (96 mg, 0.15 mmol), and 4,5-dicyanoimidazole activator solution (900 μL, 0.23 mmol) in 4 mL of acetonitrile. Oxidation by addition of *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane, 42 μL, 0.23 mmol). The product (37 mg, 0.043 mmol, 29%) was obtained as a colorless wad after ion exchange. $^1$H NMR (600 MHz, MeOH-$d_4$): $\delta$ [ppm] = 7.71 (s, 1H, H-6); 7.42−7.40 (m, 4H, H-c, H-c′); 7.07−7.05 (m, 4H, H-d, H-d′); 6.20 (dd, $^3J_{H,H}$ = 6.7 Hz, $^3J_{H,H}$ = 6.7 Hz, 1H, H-1′); 5.13−5.11 (m, 4H, H-a, H-a′); 4.40−4.38 (m, 1H, H-3′); 4.21−4.18 (m, 1H, H-5′a); 4.13−4.09 (m, 1H, H-5′b); 4.03 (bs, 1H, H-4′); 2.59−2.56 (m, 4H, H-g, H-g′); 2.36−2.31 (m, 2H, H-2′a); 2.30−2.26 (m, 2H, H-2′b); 1.90 (s, 3H, H-7); 1.72 (dt, $^3J_{H,H}$ = 7.6 Hz, $^3J_{H,H}$ = 7.6 Hz 4H, H-h, H-h′); 1.48−1.29 (m, 10H, H-i, H-i′−H-l′); 0.98 (t, $^3J_{H,H}$ = 7.4 Hz, 3H, H-j); 0.92 (t, $^3J_{H,H}$ = 6.6 Hz, 3H, H-m′). $^{13}$C NMR (150 MHz, MeOH-$d_4$): $\delta$ [ppm] = 173.7 (C-f, C-f′); 166.4 (C-4); 152.5 (C-e, Ce′); 152.3 (C-2); 137.8 (C-6); 134.9 (C-b/C-b′); 134.8 (C-b/C-b′); 130.5 (C-c, C-c′); 122.9 (C-d, C-d′); 112.2 (C-5); 85.8 (C-1′); 84.4 (d, $^3J_{C,P}$ = 9.4 Hz, C-4′); 70.4 (d, $^2J_{C,P}$ = 5.6 Hz, C-a/C-a′); 67.2 (d, $^3J_{C,P}$ = 6.1 Hz, C-5′); 62.5 (C-3′); 38.0 (C-2′); 35.0 (C-g/C-g′); 34.8 (C-g/c-g′); 32.9, 30.1, 30.1, 23.7 (C-i′−C-l′); 28.1 (C-h, C-h′); 26.0 (C-h, C-h′); 23.2 (C-i) 14.4 (C-m′); 14.1 (C-j); 12.6 (C-7). $^1$H NMR (600 MHz, MeOH-$d_4$): $\delta$ [ppm] = 7.71 (s, 1H, H-6); 7.42−7.40 (m, 4H, H-c, H-c′); 7.07−7.05 (m, 4H, H-d, H-d′); 6.20 (dd, $^3J_{H,H}$ = 6.7 Hz, $^3J_{H,H}$ = 6.7 Hz, 1H, H-1′); 5.13−5.11 (m, 4H, H-a, H-a′); 4.40−4.38 (m, 1H, H-3′); 4.21−4.18 (m, 1H, H-5′a); 4.13−4.09 (m, 1H, H-5′b); 4.03 (bs, 1H, H-4′); 2.59−2.56 (m, 4H, H-g, H-g′); 2.36−2.31 (m, 2H, H-2′a); 2.30−2.26 (m, 2H, H-2′b); 1.90 (s, 3H, H-7); 1.72 (dt, $^3J_{H,H}$ = 7.6 Hz, $^3J_{H,H}$ = 7.6 Hz 4H, H-h, H-h′); 1.48−1.29 (m, 10H, H-i, H-i′−H-l′); 0.98 (t, $^3J_{H,H}$ = 7.4 Hz, 3H, H-j); 0.92 (t, $^3J_{H,H}$ = 6.6 Hz, 3H, H-m′). HRMS (ESI$^+$, $m/z$) calcd for $C_{37}H_{49}N_5O_{14}P_2Na$: 872.2643 [M + Na]$^+$. Found: 872.2568.

**Ammonium-$C_4H_9$/$C_9H_{19}$-Di*PP*ro-AZTDP 10b.** General procedure E; phosphoramidite **5g** (117 mg, 0.191 mmol), AZT monophosphate (79 mg, 0.13 mmol), and 4,5-dicyanoimidazole activator solution (760 μL, 0.19 mmol) in 3 mL of acetonitrile. Oxidation by addition of *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane, 35 μL, 0.91 mmol). The product (97 mg, 0.11 mmol, 85%) was obtained as a colorless wad after ion exchange. $^1$H NMR (600 MHz, MeOH-$d_4$): $\delta$ [ppm] = 7.72 (s, 1H, H-6); 7.42−7.40 (m, 4H, H-c, H-c′); 7.06−7.05 (m, 4H, H-d, H-d′); 6.21 (dd, $^3J_{H,H}$ = 6.8 Hz, $^3J_{H,H}$ = 6.8 Hz, 1H, H-1′); 5.14−5.11 (m, 4H, H-a, H-a′); 4.40 (dt, $^3J_{H,H}$ = 6.7 Hz, $^3J_{H,H}$ = 6.8 Hz, 1H, H-3′); 4.21−4.18 (m, 1H, H-5′a); 4.13−4.10 (m, 1H, H-5′b); 4.04−4.03 (m, 1H, H-4′); 2.59−2.56 (m, 4H, H-g, H-g′); 2.36−2.31 (m, 2H, H-2′a); 2.30−2.26 (m, 2H, H-2′b); 1.90 (s, 3H, H-7); 1.72 (dt, $^3J_{H,H}$ = 7.4 Hz, $^3J_{H,H}$ = 7.6 Hz 4H, H-h, H-h′); 1.48−1.29 (m, 14H, H-i, H-i′−H-n′); 0.99 (t, $^3J_{H,H}$ = 7.4 Hz, 3H, H-j); 0.91 (t, $^3J_{H,H}$ = 6.8 Hz, 3H, H-o′). $^{13}$C NMR (150 MHz, MeOH-$d_4$): $\delta$ [ppm] = 173.7 (C-f, C-f′); 166.5 (C-4); 152.5 (C-e, Ce′); 152.4 (C-2); 137.8 (C-6); 134.8 (C-b′, C-b); 130.5 (C-c, C-c′) 122.9 (C-d, C-d′); 112.2 (C-5); 85.8 (C-1′); 84.4 (d, $^3J_{C,P}$ = 8.8 Hz, C-4′); 70.4 (d, $^2J_{C,P}$ = 5.5 Hz, C-a/C-a′); 67.3 (d, $^3J_{C,P}$ = 5.5 Hz, C-5′); 62.5 (C-3′); 38.0 (C-2′); 35.0 (C-g/C-g′); 34.8 (C-g/c-g′); 33.0, 30.6, 30.4, 30.2, 23.7 (C-i′−C-n′); 28.1 (C-h, C-h′); 26.0 (C-h, C-h′); 23.3 (C-i); 14.4 (C-o′); 14.1 (C-j); 12.6 (C-7). $^{31}$P NMR (243 MHz, MeOH-$d_4$, decoupled): $\delta$/ppm = −12.16 (d, $^2J_{P,P}$ = 20.6 Hz, 1P, $P_\beta$); −12.78 (d, $^2J_{P,P}$ = 21.7 Hz,



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J. Med. Chem. 2015, 58, 6114−6130

1P, $P_\alpha$). HRMS (ESI$^+$, $m/z$) calcd for $C_{39}H_{53}N_5O_{14}P_2Na$: 900.2962 $[M + Na]^+$. Found: 900.2946. RP18-HPLC: method B, $t_R$ = 20.5 min.

**Ammonium-$C_4H_9$/$C_{11}H_{23}$-Di*PP*ro-AZTDP 10c.** General procedure E; phosphoramidite **5h** (120 mg, 0.17 mmol), AZT monophosphate (69 mg, 0.11 mmol), and 4,5-dicyanoimidazole activator solution (550 μL, 0.14 mmol) in 3 mL of acetonitrile. Oxidation by addition of *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane, 31 μL, 0.17 mmol). The product (37 mg, 0.040 mmol, 37%) was obtained as a colorless wad after ion exchange. $^1$H NMR (600 MHz, MeOH-$d_4$): δ [ppm] = 7.75 (s, 1H, H-6); 7.21−7.12 (m, 4H, H-c, H-c′); 6.85−6.84 (m, 4H, H-d, H-d′); 6.00 (dd, $^3J_{H,H}$ = 6.8 Hz, $^3J_{H,H}$ = 6.8 Hz, 1H, H-1′); 4.92−4.90 (m, 4H, H-a, H-a′); 4.19 (dt, $^3J_{H,H}$ = 6.6 Hz, $^3J_{H,H}$ = 6.8 Hz, 1H, H-3′); 4.00−3.97 (m, 1H, H-5′a); 3.92−3.89 (m, 1H, H-5′b); 3.83−3.82 (m, 1H, H-4′); 2.38−2.35 (m, 4H, H-g, H-g′); 2.15−2.10 (m, 2H, H-2′a); 2.08−2.05 (m, 2H, H-2′b); 1.69 (s, 3H, H-7); 1.55−1.48 (m, 4H, H-h, H-h′); 1.28−1.08 (m, 18H, H-i, H-i′−H-p′); 0.78 (t, $^3J_{H,H}$ = 7.4 Hz, 3H, H-j); 0.69 (t, $^3J_{H,H}$ = 6.9 Hz, 3H, H-q′). $^{13}$C NMR (150 MHz, MeOH-$d_4$): δ [ppm] = 173.3 (C-f, C-f′); 166.4 (C-4); 152.5 (C-e, Ce′); 152.4 (C-2); 137.8 (C-6); 134.9 (C-b′, C-b); 130.5 (d, C-c, C-c′); 122.9 (C-d, C-d′); 112.1 (C-5); 85.8 (C-1′); 84.4 (d, $^3J_{C,P}$ = 9.4 Hz, C-4′); 70.3 (d, $^2J_{C,P}$ = 5.5 Hz, C-a/C-a′); 67.3 (d, $^3J_{C,P}$ = 5.9 Hz, C-5′); 62.5 (C-3′); 38.0 (C-2′); 35.0 (C-g/c-g′); 34.8 (C-g/c-g′); 33.1, 30.7, 30.6, 30.5, 30.4, 30.2, 23.3 (C-i,C-i′−C-p′); 28.1 (C-h, C-h′); 26.0 (C-h, C-h′); 14.1 (C-j); 14.5 (C-q′); 12.6 (C-7). $^{31}$P NMR (243 MHz, MeOH-$d_4$, decoupled): δ/ppm = −12.16 (d, $^2J_{P,P}$ = 20.3 Hz, 1P, $P_\beta$); −12.78 (d, $^2J_{P,P}$ = 20.4 Hz, 1P, $P_\alpha$). HRMS (ESI$^+$, $m/z$) calcd for $C_{41}H_{57}N_5O_{14}P_2Na$: 928.3275 $[M + Na]^+$. Found: 928.3252. RP18-HPLC: method B, $t_R$ = 21.9 min.

**Ammonium-$C_4H_9$/$CF_3$-Ph-Di*PP*ro-AZTDP 10d.** General procedure E; phosphoramidite **5i** (179 mg, 0.282 mmol), AZT monophosphate (117 mg, 0.188 mmol), and 4,5-dicyanoimidazole activator solution (1.13 mL, 0.282 mmol) in 5 mL of acetonitrile. Oxidation by addition of *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane, 51 μL, 0.282 mmol). The product (80 mg, 0.088 mmol, 47%) was obtained as a colorless wad after ion exchange. $^1$H NMR (600 MHz, MeOH-$d_4$): δ [ppm] = 8.38−8.36 (m, 2H, H-h′/H-i′); 7.90−7.89 (m, 2H, H-h′/H-i′); 7.73 (s, 1H, H-6); 7.49−7.47 (m, 2H, H-c′); 7.44−7.41 (m, 2H, H-c); 7.25−7.23 (m, 2H, H-d′); 7.08−7.06 (m, 2H, H-d); 6.22 (dd, $^3J_{H,H}$ = 6.8 Hz, $^3J_{H,H}$ = 6.8 Hz, 1H, H-1′); 5.18−5.13 (m, 4H, H-a, H-a′); 4.43−4.41 (m, 1H, H-3′); 4.23−4.21 (m, 1H, H-5′a); 4.15−4.12 (m, 1H, H-5′b); 4.06−4.05 (m, 1H, H-4′); 2.56 (t, $^3J_{H,H}$ = 7.4 Hz, 2H, H-g); 2.37−2.32 (m, 1H, H-2′a); 2.31−2.27 (m, 1H, H-2′b); 1.90 (s, 3H, H-7); 1.68 (dt, $^3J_{H,H}$ = 7.4 Hz, $^3J_{H,H}$ = 7.6 Hz, 2H, H-h); 1.43 (dq, $^3J_{H,H}$ = 7.5 Hz, $^3J_{H,H}$ = 7.5 Hz, 2H, H-i); 0.96 (t, $^3J_{H,H}$ = 7.4 Hz, 2H, H-j). $^{13}$C NMR (150 MHz, MeOH-$d_4$): δ [ppm] = 173.6 (C-f); 166.3 (C-4); 166.3 (C-f′); 152.4 (C-2); 152.3 (C-e); 152.2 (C-e′); 137.4 (C-6); 135.9 (C-j′); 135.3 (C-b′); 134.8 (C-b); 134.3 (C-g′); 131.6 (C-h′/C-i′); 130.6 (d, $^4J_{C,P}$ = 4.2 Hz, C-c′); 130.1 (d, $^4J_{C,P}$ = 2.5 Hz, C-c); 126.5 (C-h′/C-i′); 124.3 ($CF_3$); 122.6 (C-d′); 122.6 (C-d); 112.1 (C-5); 85.4 (C-1′); 84.4 (d, $^3J_{C,P}$ = 9.2 Hz, C-4′); 70.4 (2 ×d, $^2J_{C,P}$ = 6.3 Hz, $^2J_{C,P}$ = 4.9 Hz C-a, C-a′); 67.3 (d, $^3J_{C,P}$ = 5.6 Hz, C-5′); 62.2 (C-3′); 37.8 (C-2′b); 37.7 (C-2′a); 34.4 (C-g); 27.7 (C-h); 22.9 (C-i); 14.1 (C-j); 12.4 (C-7). $^{19}$F NMR (565 MHz, MeOH-$d_4$): δ/ppm = −65.7. $^{31}$P NMR (243 MHz, MeOH-$d_4$, decoupled): δ/ppm = −12.14 (d, $^2J_{P,P}$ = 20.2 Hz, 1P, $P_\beta$); −12.67−12.81 (m, 1P, $P_\alpha$). HRMS (ESI$^+$, $m/z$) calcd for $C_{37}H_{39}F_3N_5O_{14}P_2$: 896.1921 $[M + H]^+$. Found: 896.1914. RP18-HPLC: method B, $t_R$ = 18.0 min.

**Ammonium-$C_4H_9$-Di*PP*ro-AZTDP 11a.** General procedure E; symmetric bis(4-pentanoyloxymethyl)-*N*,*N*-diisopropylamino-phosphoramidite (133 mg, 0.243 mmol), AZT monophosphate (101 mg, 0.162 mmol), and 4,5-dicyanoimidazole activator solution (970 μL, 0.243 mmol) in 4 mL of acetonitrile. Oxidation by addition of *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane, 44 μL, 0.243 mmol). The product (84 mg, 0.10 mmol, 63%) was obtained as a colorless wad after ion exchange. $^1$H NMR (600 MHz, MeOH-$d_4$): δ [ppm] = 7.72 (s, 1H, H-6); 7.42−7.40 (m, 4H, H-c); 7.07−7.05 (m, 4H, H-d); 6.21 (dd, $^3J_{H,H}$ = 6.8 Hz, $^3J_{H,H}$ = 6.8 Hz, 1H, H-1′); 5.14−5.11 (m, 4H, H-a); 4.41−4.39 (m, 1H, H-3′); 4.21−4.18 (m, 1H, H-5′a); 4.13−4.10 (m, 1H, H-5′b); 4.04−4.03 (m, 1H, H-4′); 2.58 (t, $^3J_{H,H}$ = 7.4 Hz, 2H, H-g); 2.36−2.26 (m, 2H, H-2′); 1.90 (s, 3H, H-7); 1.74−1.69 (m 2H, H-h); 1.49−1.43 (m, 2H, H-i); 0.99 (t, $^3J_{H,H}$ = 7.4 Hz, 6H, H-j). $^{13}$C NMR (150 MHz, MeOH-$d_4$): δ [ppm] = 173.7 (C-f); 166.4 (C-4); 152.5 (C-e); 152.3 (C-2); 137.8 (C-6); 134.9 (C-b); 130.5 (d, $^4J_{C,P}$ = 2.3 Hz, C-c); 122.9 (C-d′); 122.6 (C-d); 112.2 (C-5); 85.8 (C-1′); 84.4 (d, $^3J_{C,P}$ = 9.0 Hz, C-4′); 70.3 (d, $^2J_{C,P}$ = 6.1 Hz C-a); 67.3 (d, $^3J_{C,P}$ = 5.8 Hz, C-5′); 62.5 (C-3′); 38.0 (C-2′); 34.8 (C-g); 28.1 (C-h); 23.3 (C-i); 14.1 (C-j); 12.6 (C-7). $[\alpha]_D^{23}$ 7 (*c* 0.1 mg/mL, $CH_3OH$). $^{31}$P NMR (243 MHz, MeOH-$d_4$, decoupled): δ/ppm = −12.2 (d, $^2J_{P,P}$ = 20.4 Hz, $P_\beta$); −12.8 (d, $^2J_{P,P}$ = 20.5 Hz, $P_\alpha$). HRMS (ESI$^+$, $m/z$) calcd for $C_{34}H_{44}N_5O_{14}P_2$: 808.2355 $[M + H]^+$. Found: 808.2352. RP18-HPLC: method B, $t_R$ = 17.4 min.

**Ammonium-$C_6H_{13}$-Di*PP*ro-AZTDP 11b.** General procedure E; symmetric bis(4-heptanoyloxymethyl)-*N*,*N*-diisopropylamino-phosphoramidite (217 mg, 0.36 mmol), AZT monophosphate (150 mg, 0.24 mmol), and 4,5-dicyanoimidazole activator solution (1.44 mL, 0.36 mmol) in 6 mL of acetonitrile. Oxidation by addition of *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane, 66 μL, 0.36 mmol). The product (83 mg, 0.09 mmol, 39%) was obtained as a colorless wad after ion exchange. $^1$H NMR (600 MHz, MeOH-$d_4$): δ [ppm] = 7.72 (s, 1H, H-6); 7.42−7.40 (m, 4H, H-c); 7.07−7.05 (m, 4H, H-d); 6.21 (dd, $^3J_{H,H}$ = 6.8 Hz, $^3J_{H,H}$ = 6.8 Hz, 1H, H-1′); 5.14−5.11 (m, 4H, H-a); 4.41−4.39 (m, 1H, H-3′); 4.21−4.18 (m, 1H, H-5′a); 4.13−4.10 (m, 1H, H-5′b); 4.03 (bs, 1H, H-4′); 2.58 (t, $^3J_{H,H}$ = 7.4 Hz, 2H, H-g); 2.36−2.26 (m, 2H, H-2′); 1.90 (s, 3H, H-7); 1.75−1.70 (m 2H, H-h); 1.44−1.41 (m, 2H, H-i); 1.37−1.36 (m, 8H, H-j, H-k); 0.93 (t, $^3J_{H,H}$ = 6.6 Hz, 6H, H-l). $^{13}$C NMR (150 MHz, MeOH-$d_4$): δ [ppm] = 173.7 (C-f); 166.4 (C-4); 152.5 (C-e); 152.4 (C-2); 137.8 (C-6); 134.9 (C-b); 130.5 (d, $^4J_{C,P}$ = 2.2 Hz, C-c); 122.9 (C-d′); 122.6 (C-d); 112.2 (C-5); 85.8 (C-1′); 84.4 (d, $^3J_{C,P}$ = 9.1 Hz, C-4′); 70.3 (d, $^2J_{C,P}$ = 6.0 Hz C-a); 67.3 (d, $^3J_{C,P}$ = 6.1 Hz, C-5′); 62.5 (C-3′); 38.0 (C-2′); 35.0 (C-g); 32.7 (C-j/C-k); 29.9 (C-i); 25.9 (C-h); 23.6 (C-j/C-k); 14.4 (C-l); 12.6 (C-7). $[\alpha]_D^{23}$ 12 (*c* 0.1 mg/mL, $CH_3OH$). $^{31}$P NMR (243 MHz, MeOH-$d_4$, decoupled): δ/ppm = −12.2 (d, $^2J_{P,P}$ = 20.9 Hz, $P_\beta$); −12.8 (d, $^2J_{P,P}$ = 21.4 Hz, $P_\alpha$). HRMS (ESI$^+$, $m/z$) calcd for $C_{38}H_{52}N_5O_{14}P_2$: 864.2991 $[M + H]^+$. Found: 864.2978. RP18-HPLC: method B, $t_R$ = 20.0 min.

**Ammonium-$C_9H_{19}$-Di*PP*ro-AZTDP 11c.** General procedure E; symmetric bis(4-decanoyloxymethyl)-*N*,*N*-diisopropylamino-phosphoramidite (120 mg, 0.18 mmol), AZT monophosphate (72 mg, 0.12 mmol), and 4,5-dicyanoimidazole activator solution (720 μL, 0.18 mmol) in 3 mL of acetonitrile. Oxidation by addition of *tert*-butyl hydroperoxide (5.5 M solution in *n*-decane, 33 μL, 0.18 mmol). The product (58 mg, 0.060 mmol, 50%) was obtained as a colorless wad after ion exchange. $^1$H NMR (600 MHz, MeOH-$d_4$): δ [ppm] = 7.72 (s, 1H, H-6); 7.42−7.39 (m, 4H, H-c); 7.06−7.04 (m, 4H, H-d); 6.21 (dd, $^3J_{H,H}$ = 6.8 Hz, $^3J_{H,H}$ = 6.8 Hz, 1H, H-1′); 5.13−5.11 (m, 4H, H-a); 4.41−4.39 (m, 1H, H-3′); 4.21−4.18 (m, 1H, H-5′a); 4.13−4.10 (m, 1H, H-5′b); 4.04 (bs, 1H, H-4′); 2.57 (t, $^3J_{H,H}$ = 7.4 Hz, 4H, H-g); 2.36−2.29 (m, 4H, H-2′); 1.90 (s, 3H, H-7); 1.76−1.71 (m 4H, H-h); 1.43−1.31 (m, 24H, H-i - H-n); 0.91 (t, $^3J_{H,H}$ = 6.8 Hz, 6H, H-o). $^{13}$C NMR (150 MHz, MeOH-$d_4$): δ [ppm] = 173.7 (C-f); 166.5 (C-4); 152.6 (C-e); 152.5 (C-2); 137.8 (C-6); 134.9 (C-b); 130.5 (C-c); 122.9 (C-d); 112.2 (C-5); 85.8 (C-1′); 84.4 (d, $^3J_{C,P}$ = 9.5 Hz, C-4′); 70.4 (d, $^2J_{C,P}$ = 5.8 Hz C-a); 67.3 (d, $^3J_{C,P}$ = 5.9 Hz, C-5′); 62.5 (C-3′); 38.0 (C-2′); 35.0 (C-g); 33.1, 30.6, 30.4, 30.2, 23.7 (C-i - C-n); 26.0 (C-h); 14.4 (C-o); 12.6 (C-7). $[\alpha]_D^{23}$ 37 (*c* 0.1 mg/mL, $CH_3OH$). $^{31}$P NMR (243 MHz, MeOH-$d_4$, decoupled): δ/ppm = −12.2 (d, $^2J_{P,P}$ = 20.4 Hz, $P_\beta$); −12.8 (d, $^2J_{P,P}$ = 20.4 Hz, $P_\alpha$). HRMS (ESI$^+$, $m/z$) calcd for $C_{44}H_{64}N_5O_{14}P_2$: 948.3930 $[M + H]^+$. Found: 948.3915. RP18-HPLC: method B, $t_R$ = 24.4 min.

**Compounds Evaluation Methods. Hydrolysis Studies in Phosphate Buffer at pH 7.3.** Hydrolysis was started by addition of 300 μL of 50 mM phosphate buffer (547 mg (3.85 mol), disodium hydrogen phosphate, and 155 mg (0.890 mol) of potassium dihydrogen phosphate, filling up with water to 100 mL. The pH was adjusted by addition of NaOH or phosphoric acid to 300 μL of 1.9 mM solution of the Di*PP*ro-NDP in DMSO. The hydrolysis solution was incubated at 37 °C. Aliquots were taken at certain points of time and frozen in liquid nitrogen. Directly after unfreezing analysis was followed by analytical RP-18-HPLC.



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**Hydrolysis Studies in CEM/0 Cell Extracts.** An amount of 50 μL CEM/0 cell extracts was mixed with 10 μL of water. By addition of 10 μL of a 6 mM solution of the Di*PP*ro-NDP in DMSO, the hydrolysis was started. Six to eight of these samples were prepared and incubated at 37 °C for different periods of time. Hydrolysis was stopped by quenching with 150 μL of methanol. After 5 min on ice, 5 min in an ultrasonic bath, and centrifugation at 13 000 rpm (Heraeus, Biofuge Pico) the samples were filtered and frozen in liquid nitrogen until an amount of 35 μL was injected into the analytical RP-18-HPLC instrument. Hydrolysis was monitored for a maximum of 10 h.

**Hydrolysis Studies in RPMI Medium.** An amount of 50 μL of RPMI-culture medium without FCS was mixed with 10 μL of water. By addition of 10 μL of a 6 mM solution of the Di*PP*ro-NDP in DMSO, the hydrolysis was started. Six to eight of these samples were prepared and incubated at 37 °C for different periods of time. After a period of time an amount of 150 μL of methanol was added and the sample was frozen in liquid nitrogen until an amount of 35 μL was injected into the analytical RP-18-HPLC instrument.

**Hydrolysis Studies with PLE.** An amount of 10 μL of a 6 mM solution of the Di*PP*ro-NDP in DMSO was mixed with 15 μL of DMSO and 100 μL of 50 mM phosphate buffer. By addition of 7.5 μL of PLE solution (3 mg/mL in PBS, 17 units/mg), the hydrolysis was started. Hydrolysis was stopped by quenching with 132.5 μL of methanol. After 5 min on ice, 5 min in an ultrasonic bath, and centrifugation at 13 000 rpm (Heraeus, Biofuge Pico) the samples were filtered and frozen in liquid nitrogen until an amount of 35 μL was injected into the analytical RP-18-HPLC instrument.

**Preparation of CEM Cell Extracts.** Human $CD4^+$ T-lymphocyte CEM cells were grown in RPMI-1640-based cell culture medium to a final density of about $3 \times 10^6$ cells/mL. After centrifugation for 10 min at 1250 rpm (Heraeus, Megafuge 3.0R) at 4 °C, washing twice with cold phosphate buffered saline (PBS), the pellet was resuspended at $10^8$ cells/mL and sonicated three times for 10 s. After a second centrifugation of this suspension at 10 000 rpm (Heraeus, Megafuge 3.0R) the supernatant was divided into aliquots and frozen to −80 °C until used.

**Antiviral Assay.** Inhibition of HIV-1($III_B$)- and HIV-2(ROD)-induced cytopathogenicity in wild-type CEM/0 or thymidine kinase-deficient $CEM/TK^-$ cell cultures was measured in microtiter 96-well plates containing ∼$3 \times 10^5$ CEM cells/mL infected with 100 $CCID_{50}$ of HIV per milliliter and containing appropriate dilutions of the test compounds. After 4−5 days of incubation at 37 °C in a $CO_2$-controlled humidified atmosphere, CEM giant (syncytium) cell formation was examined microscopically. $EC_{50}$ (50% effective concentration) was defined as the compound concentration required to inhibit HIV-induced giant cell formation by 50%.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

A csv file containing molecular formula strings. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b00737.

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### Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS USED

AZT, 3′-azido-3′-deoxythymidine; d4T, 3′-deoxy-2′,3′-didehydrothymidine; DCI, 4,5-dicyanoimidazole; DIPA, *N*,*N*-diisopropylamine; FCS, fetal calf serum; HILIC, hydrophilic interaction liquid chromatography; NDP, nucleoside diphosphate; NMP, nucleoside monophosphate; PLE, pig live resterase; $t_R$, retention time; RP, reversed phase; TK, thymidine kinase

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